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Effects of low crude-protein diets fortified with crystalline amino acids on growth performance and nitrogen retention of broiler chicks

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Effects of low crude-protein diets fortified with crystalline amino acids on growth performance and nitrogen retention of broiler chicks

by

Kristjan Bregendahl

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For the Graduate College

To my grandparents, Esther and Poul Nørgaard,

who taught me the importance of all things in nature,

inspiring me to pursue a life in science.

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LIST OF ABBREVIATIONS

ADFI	Average daily feed intake
ADG	Average daily gain
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
Asn	Asparagine
ATP	Adenosine triphosphate
BV	Biological value
BW	Body weight
cAA	Crystalline amino acids
Cl	Chloride
CP	Crude protein
CP_{EAA}:CP_{Total}	Ratio of crude protein from essential amino acids to total crude protein
d	Day
dEB	Dietary electrolyte balance
DFMO	Difluoromethylornithine
DM	Dry matter
DNA	Deoxyribonucleic acid
EAA	Essential amino acid
G:F	Gain-to-feed ratio; feed utilization
Gln	Glutamine
h	Hour
H	Hydrogen
K	Potassium

kcal	Kilocalories
L	Linear contrast
LSD	Least significant difference
Mcal	Megacalories
ME	Metabolizable energy
ME_n	Nitrogen-corrected metabolizable energy
Min	Minute
mM	Millimolar
N	Nitrogen
Na	Sodium
NE	Net energy
NEAA	Nonessential amino acid
NRC	National Research Council
ODC	Ornithine decarboxylase
P	Probability
PER	Protein efficiency ratio
RNA	Ribonucleic acid
Q	Quadratic contrast
SAM	S-Adenosinemethionine
SBM	Soybean meal
SE	Standard error
TAC	Triammonium citrate
TCA cycle	Tricarboxylic acid or Krebs cycle
V_{Max}	Maximal rate
vs	Versus
wk	Week

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ABSTRACT

Four growth performance and nitrogen (N) retention trials were conducted to investigate potential reasons for the relatively poor performance of broiler chicks fed low crude protein (CP) diets. Male chicks (1-d-old) were fed a common corn–soybean (SBM) diet (23% CP) for 7 d and subsequently allotted to treatment diets in a completely randomized design (10 chicks per floor pen, 6 replications). Chicks had free access to the isoenergetic diets (3,200 kcal ME_n/kg) for 2 wk, after which chicks were weighed, fasted for 24 h, and the whole-body N contents of two chicks per pen (and six baseline chicks) determined. In Trial 1, dietary treatments consisted of an intact-protein diet (corn–SBM, 24% CP) and a similar diet, in which half the intact protein was replaced on a true digestible basis with all essential and all nonessential amino acids (EAA and NEAA, respectively) in crystalline form. In subsequent trials, corn–SBM diets (23% CP) served as control diets and low-CP diets were formulated by partially replacing SBM with corn and crystalline arginine, isoleucine, lysine, methionine, threonine, and valine to meet 105% of NRC (1994) recommended EAA concentrations. In Trial 2, glutamine or asparagine replaced 1% triammonium citrate in the low-CP diet (19% CP). In Trials 3 and 4, dietary concentrations of crystalline EAA and NEAA, respectively, were increased incrementally in the low-CP diets (19 to 20% CP). Chicks fed the intact-protein diet (Trial 1) grew faster, utilized feed more efficiently, and retained more N ($P<0.05$) than did chicks fed the free amino acid (AA) diet, notwithstanding equal dietary concentrations of true digestible AA. In subsequent trials, chicks fed low-CP diets grew slower, utilized feed less efficiently, and retained less N than chicks fed the control diets ($P<0.05$), despite additions of crystalline glutamine or asparagine, and despite increased dietary concentrations of crystalline EAA and/or NEAA. The growth performance and N retention of chicks fed diets containing large amounts of free AA were inferior to those of chicks fed intact-protein diets. Consequently, dietary free AA may not be utilized as well for growth and protein deposition as AA originating from intact protein.

LITERATURE REVIEW

Introduction

Corn and soybean meal (SBM) are included in most pig and broiler diets as sources of protein. When corn and SBM are used to supply all of the protein, the diet contains amino acids in excess of the pigs' or broilers' requirements. The balance of amino acids in a given corn–SBM diet is different from that needed by the animal because sufficient protein must be added to meet or exceed the requirement not just for one, but for all essential amino acids. The relatively high levels of amino acids results in a diet that meets the requirement for the first-limiting amino acid, while all other amino acids necessarily exceed their respective requirements. Dietary amino acids that are supplied in excess of the requirements cannot be stored in the body and are instead transaminated and/or deaminated, with the majority of the excess nitrogen (N) excreted in the urine as urea (in pigs) or uric acid (in poultry). The carbon skeletons of excess amino acids are converted into glucose, fatty acids, ketone bodies, and other compounds or are excreted as CO_2 after complete oxidation in the tricarboxylic acid (TCA) cycle (Riis, 1983; Voet and Voet, 1995). Jungas et al. (1992) argued that the energy derived from complete oxidation of 'surplus' amino acids (i.e., those not used for protein synthesis) would exceed the adenosine triphosphate (ATP) needs of the tissues. Therefore, even if amino acids are catabolized rather than used for protein deposition, they are not necessarily completely oxidized to CO_2 and H_2O, but only catabolized to the extent that their glucogenic potential is preserved. Glucose and/or lipid synthesis from carbon skeletons of dietary amino acids thus occur in the fed and early post-absorptive state (Stipanuk and Watford, 2000). Catabolism of amino acids takes place in the liver, although the branched-chained amino acids (i.e., leucine, isoleucine, and valine) are primarily degraded in muscle (Lindsay, 1976; Groff and Gropper, 2000). A significant catabolism of amino acids also takes place in the enterocytes (Windmueller and Spaeth, 1980; Stoll et al.,

1998; Reeds et al., 2000). The excess dietary protein not only is wasteful, but also represents an economic loss to the farmer (although some excreted N can be reclaimed through manure application in lieu of chemical fertilizers). Moreover, the excreted N also contributes to offensive odors and environmental pollution. The growing concern of increased N emissions from livestock has been addressed by a combination of 1) adjusting the dietary content of amino acids (protein) to animals' requirements at a given age (i.e., 'phase feeding') and 2) by lowering the amount of dietary crude protein (CP) with the use of crystalline amino acids (Aumüller, 1991; Hobbs et al., 1996; Jongbloed and Lenis, 1998).

Because of the excess amino acids in corn–SBM diets, the CP content of the diet is relatively high. It is possible to lower the CP content of a given pig or broiler diet and still meet established amino acid requirements by replacing part of the intact protein with crystalline amino acids, thereby obtaining a balance of dietary amino acids closer to the animal's requirements. In other words, the dietary protein becomes closer to an 'ideal protein.' Not only can the cost of the diet be lowered (depending on the relative cost of corn, SBM, and crystalline amino acids) by feeding low-CP diets, but the amount of excreted N (including NH_3) can be lowered (Canh et al., 1998; Ferguson et al., 1998; Jongbloed and Lenis, 1998), potentially decreasing offensive odors and pollution. However, there are numerous studies showing that growth performance and/or carcass quality in pigs (e.g., Kerr et al., 1995; Tuitoek et al., 1997; Knowles et al., 1998) and poultry (e.g., Ferguson et al., 1998; Aletor et al., 2000; Leeson et al., 2000) decrease if the dietary CP content is more than three to four percentage points below National Research Council (NRC) recommended concentrations. These adverse effects occur even though the low-CP diets meet recommended amino acid concentrations and ratios of essential-to-nonessential amino acids. No significant effects are observed on growth performance and/or body composition if the dietary CP content is lowered by only two percentage points.

Therefore, it is generally recommended not to lower the dietary CP content (using crystalline amino acids) by more than two to three percentage points (Kornegay and Verstegen, 2001; Lewis, 2001).

Attempts to improve the growth performance of animals fed low-CP diets, reviewed below, have failed; it is currently unknown why animals fed low-CP diets do not perform well. Not all studies with low-CP diets (e.g., Han et al., 1992; Canh et al., 1998; Kendall et al., 1999) show an inferior growth performance, yet, the reason for this ambiguity is not known. Until the cause or causes for the poor utilization of low-CP diets can be determined and corrected, the protein concentration in practical diets will remain high with subsequent potential adverse economic and/or environmental implications.

Net energy

Low-CP diets contain a dietary supply of amino acids close to ideal (i.e., few excess amino acids), and the animals may therefore have a decreased need for deamination reactions with concomitant decreased formation of urea or uric acid. Furthermore, organ weights of animals fed low-CP diets may decrease (Kerr et al., 1995), potentially decreasing the requirement for maintenance energy. Unless the dietary energy concentrations are adjusted accordingly, the decreased need for (maintenance) energy by animals fed low-CP diets may result in increased carcass fatness, which is further augmented by a somewhat higher dietary net energy (NE) content in low-CP diets compared with high-CP diets. Low-CP diets are inherently higher in NE, because corn, which replaces SBM in the low-CP diet, contains considerably more NE than does SBM (De Groote, 1974; NRC, 1998).

Only a few studies have investigated the influence of energy concentration in low-CP diets on growth performance and body composition. Kendall et al. (1999) added 10% soybean hulls to a low-CP diet (9.7% CP) fed to finishing pigs and compared backfat depths and growth

performance with pigs fed a diet containing 12.4% CP. The soybean hulls lowered the dietary NE and should have corrected the poor performance observed with low-CP diets. No effect of the dietary treatments, however, was observed ($P > 0.10$) in two of the four replications, while the low-CP diet resulted in a slower ($P < 0.05$) average daily gain (ADG) and a decreased average daily feed intake (ADFI) in the other two. Wheat middlings, with their low NE content (NRC, 1998), were added to low-CP diets for growing–finishing pigs in lieu of corn and SBM by Shriver et al. (1999). Growth performance and carcass characteristics were compared with that of pigs fed either a high- or a low-CP diet without wheat middlings. Addition of wheat middlings to the low-CP diet restored the otherwise lower ADG observed with the low-CP diet to levels obtained by feeding the high-CP diet. Although the studies by Kendall et al. (1999) and Shriver et al. (1999) indicate that growth performance may improve with the addition of fiber (and presumably with a decrease in dietary NE concentrations), neither study reported dietary NE concentrations. In contrast, Knowles et al. (1998) varied the dietary NE concentration of low-CP diets to finishing pigs by adding fiber in the form of rice hulls or wheat middlings and by changing the dietary amount of fat. In that study, the low-CP diets resulted in significantly decreased growth performance and carcass quality compared with the high-CP diet. The reduced growth performance and carcass quality occurred regardless of the dietary NE concentration and whether the change in dietary NE was through fiber or fat. Similarly, Leeson et al. (2000) found no benefit ($P > 0.05$) of formulating low-CP pullet diets on a NE basis rather than on a metabolizable energy (ME) basis. In sum, it is possible that the increased dietary NE concentration in low-CP diets is responsible for some of the decrease in growth performance and carcass quality, but other factors are more likely to influence performance.

Minimum dietary crude protein levels and nonessential amino acids

A certain amount of protein is required in the diet for growing animals to ensure optimal growth. The protein must provide both essential and nonessential amino acids, as both are needed for maintenance and protein deposition. Essential amino acids, at concentrations recommended by the NRC (1994), provide less than half the total CP for broiler chicks. The amount of total dietary CP ranges from 23 to 18% (declining with age), whereas the CP supplied by essential amino acids ranges from 10.2 to 7.9%. Correspondingly, the NRC (1998) lists a CP requirement for total CP of 26 to 13% (declining with age) of the diet, whereas the CP supplied by essential amino acids ranges from 8.4 to 3.2%. The remaining CP can be supplied as nonessential amino acids. Essential amino acids, supplied in excess of the recommended levels, will enter the pool of nonessential amino acids as they are not needed in their 'essential form' for maintenance and/or protein deposition. Hence, to meet the recommended amount of total CP, nonessential amino acids and excess essential amino acids should supply 12.8 to 10.1% CP in broiler diets and 17.6 to 9.8% CP in pig diets (depending on the growth stages). Those high concentrations of nonessential amino acids are likely not needed, but rather reflect that no dietary protein (especially the protein in corn–SBM diets, upon which the recommended levels of total-CP are based) is 'ideal protein' and that excesses of essential amino acids are difficult (expensive) to avoid in practical diets. However, the protein in low-CP diets approximate 'ideal protein' in that excesses of essential amino acids are decreased or (for some of the amino acids) completely eliminated.

Animal have a physiological need for nonessential amino acids, but, because the nonessential amino acids can be synthesized by transamination, it is impractical to define a dietary requirement for individual nonessential amino acids. Rather, an amount of nonspecific N (which may come from essential and nonessential amino acids) is needed and can be quantified through the ratio of CP from essential amino acids to total dietary CP (CP_{EAA}:CP_{Total}). Stucki

and Harper (1961) investigated the importance of the nonessential amino acid concentration on the growth performance of broiler chickens. They found that the body weight (BW) of 2 to 10 day (d) old chicks was maximized when the dietary concentration of essential amino acids was approximately double that of nonessential amino acids, corresponding to a CP_{EAA}:CP_{Total} ratio of about 0.66. However, this estimate is somewhat questionable because DL-isomers of some of the crystalline essential amino acids (isoleucine, phenylalanine, threonine, tryptophan, and valine) were used in the experiment. A more recent experiment investigating the CP_{EAA}:CP_{Total} ratio for broilers (7 to 21 d of age) was performed by Bedford and Summers (1985). Although these authors also used a mix of D- and L-isomers of some of the crystalline essential amino acids (isoleucine, threonine, and valine), the D-isomers were classified as nonessential amino acids. The optimal CP_{EAA}:CP_{Total} ratio for growth performance was approximately 0.55, regardless of CP concentration, whereas that for N retention was approximately 0.65. Thus, if a diet is formulated to contain the exact amounts of essential amino acids recommended by the NRC (1994) for 0- to 3-week (wk) old broiler chicks, the essential amino acids will supply 9.2% CP (not including the CP from proline and serine according to Bedford and Summers [1985]). Using the CP_{EAA}:CP_{Total} ratio of 0.55, the minimal concentration of dietary CP should therefore be (9.2 ÷ 0.55 =) 16.7% CP, well below the NRC-recommended concentration of 23%. By employing crystalline amino acids, it should be possible to lower the dietary CP content to approximately ([16.7 ÷ 23] × 100 =) 73% of the recommended NRC (1994) concentration for broiler chicks without affecting growth performance. It should be noted, however, that carcass protein (N retention) in the study by Bedford and Summers (1985) was directly related to the intake of essential amino acids rather than the ratio of CP_{EAA}:CP_{Total}. N retention was, therefore, maximized at the highest ratio tested, namely 0.65.

The CP_{EAA}:CP_{Total} ratio for growing pigs has also been investigated. Wang and Fuller (1989), using 25- to 55-kg pigs, concluded that for optimal N utilization, the ratio between the

dietary concentration of essential and the dietary concentration of nonessential amino acids should be 45:55, which Heger et al. (1998) translated into a CP_{EAA}:CP_{Total} ratio of approximately 0.42. Heger et al. (1998) found the CP_{EAA}:CP_{Total} ratio to be 0.48 for optimal N retention in 47-kg pigs, while the ratio was 0.66 for optimal N utilization. Lenis et al. (1999) concluded that the optimal CP_{EAA}:CP_{Total} ratio for 30- to 60-kg pigs was 0.50. Calculations from requirement tables (NRC, 1994) show that the CP contributed by essential amino acids ranges from 8.4 to 3.2% of the diet for 3- to 120-kg pigs, respectively. Applying the CP_{EAA}:CP_{Total} ratio of 0.50 (Lenis et al., 1999), it should be possible to lower the total dietary CP content to (8.4 ÷ 0.50 =) 16.9 and (3.2 ÷ 0.50 =) 6.4% total CP, respectively, without affecting growth performance.

In summary, results from pig and poultry studies indicate that the optimal CP_{EAA}:CP_{Total} ratio is approximately 0.50, meaning that half the dietary CP should come from essential and the remainder from nonessential amino acids. This finding supports the fact that protein synthesis includes both essential and nonessential amino acids at the translation level in the ribosomes and, because there are 10 of each (for pigs), the dietary protein should thus consist of approximately half essential and half nonessential amino acids.

With a minimal demand for CP from essential and nonessential amino acids established through the CP_{EAA}:CP_{Total} ratio, a low-CP diet could be too low in total dietary CP, although the concentrations of essential amino acids meet the NRC recommendations. This relationship may well explain the decreased performance observed in animals fed low-CP diets, but conflicting information exists regarding the efficacy of increasing the dietary total-CP concentration in low-CP diets by additions of nonessential, crystalline amino acids. Although neither Deschepper and De Groote (1995) nor Aletor et al. (2000) observed significant improvements in carcass quality of broiler chicks after nonessential amino acids were added to low-CP diets, Edmonds et al. (1985), as well as Fancher and Jensen (1989a,b,c), did. It is difficult to ascertain why these differences are observed, and it does not become more clear when growth performance is included

in the evaluation. Although dietary additions of glutamate did not improve carcass composition of broiler chicks fed low-CP diets in the trial by Aletor et al. (2000), growth performance was restored to control levels. In contrast, addition of nonessential amino acids to low-CP diets did not ($P < 0.05$) restore growth performance of broiler chicks in the trials by Pinchasov et al. (1990) and Fancher and Jensen (1989a,b,c). Edmonds et al. (1985) found that addition of 3% glutamate to a low-CP broiler diet improved ($P < 0.05$) growth performance, although not to control levels ($P > 0.05$).

Potentially, glutamate alone could not substitute for all the nonessential amino acids in the above-mentioned studies. Fickler and colleagues found in a series of papers (Fickler et al., 1994; Roth et al., 1994; Kirchgessner et al. 1995) that dietary omissions of individual nonessential amino acids (arginine, proline, and glutamate) decreased ($P < 0.05$) N retention and increased ($P < 0.05$) the concentrations of plasma and urinary urea in young pigs (15 kg initial BW). These responses are typical of amino acid deficient diets and imply that some nonessential amino acids may be conditionally essential. Indeed, arginine (Southern and Baker, 1983) and proline (Ball et al., 1986) are essential for young animals, but sufficient amounts of both can be synthesized from glutamate in older animals (Jones, 1985; NRC, 1994, 1998). In addition, Chung and Baker (1991) found that a mixture of the nonessential amino acids glycine, proline, and glutamate was as effective in promoting growth of pigs as was a mixture of glycine, proline, glutamate, glutamine, serine, alanine, aspartate, and asparagine in semi-synthetic diets. On the basis of these results, glutamate should be able to serve as the sole source of (added) nonessential amino acids.

If the reason for the reduced performance after feeding low-CP diets is because of a deficiency of CP (i.e., a CP_{EAA}:CP_{Total}, ratio above 0.50), performance should be unaffected until the lower CP limit is reached (i.e., when the ratio of CP_{EAA}:CP_{Total} is 0.50). Then, as the dietary CP concentration decreases (and the CP_{EAA}:CP_{Total}, ratio increases above 0.50), the performance

should decrease. In the trial by Aletor et al. (2000), total body fat and fat retention increased linearly ($P < 0.001$) with decreasing CP intake. Consequently, lowering the dietary CP concentration resulted in increasing fat content of the carcasses, even though the carcass should not have been affected until the lower CP limit was reached. Similarly, Fancher and Jensen (1989b,c) found a linear ($P < 0.05$) increase in abdominal fat deposition with decreasing dietary CP intake.

Essential amino acids

Pinchasov et al. (1990) argued that the requirements for at least some of the essential amino acids (i.e., threonine and lysine) were related directly to the dietary CP concentration. Hence, with a lowered dietary CP content, the requirement for essential amino acids should be lower. Pinchasov et al. (1990) speculated that low-CP diets result in reduced performance because of the potentially excessive amounts of dietary essential amino acids. To test this hypothesis, Pinchasov et al. (1990) formulated low-CP broiler diets with 100.0, 93.5, and 87.5% of NRC-recommended concentrations of all essential amino acids. The growth performance of broilers fed a high-CP (23% CP) control diet was superior ($P < 0.05$) to that of broilers fed low-CP diets (20 to 17% CP) containing essential amino acids at 100% of their requirements as expected by Pinchasov et al. (1990). However, lowering the contents of essential amino acids did not ($P > 0.05$) restore growth performance to that of broilers fed the control diet, nor was there a difference ($P > 0.10$) in growth performance between broilers fed the three diets with incrementally decreased concentrations of essential amino acids. The latter would indicate that the hypothesis of Pinchasov et al. (1990) was erroneous and that, perhaps, *more* essential amino acids are required in low-CP diets than recommended by the NRC. This hypothesis was investigated by Fancher and Jensen (1989a), who speculated that the NRC-recommended concentrations for some amino acids may be too low, because the recommendations were based on insufficient

experimental data. Therefore, Fancher and Jensen (1989a,c) added crystalline L-arginine, L-threonine, L-isoleucine, and L-tryptophan in excess (up to 15%) of the NRC-recommended concentrations to low-CP broiler diets. The authors speculated that the NRC levels of methionine and lysine (the first- and second-limiting amino acids for growth, respectively) were adequate, so excesses of those were not added. Although the low-CP diet resulted in lower ($P < 0.05$) growth performance and increased abdominal fat pad deposition than did the high-CP diet, additions of up to 15% above NRC concentrations of crystalline essential amino acids did not improve growth performance or carcass characteristics ($P < 0.05$). The concentrations of threonine and isoleucine (added in excess of NRC levels from crystalline sources) in plasma generally were increased ($P < 0.05$), albeit, only at the 15%-level in the case of isoleucine and methionine. Plasma arginine concentrations did not change significantly as a result of adding L-arginine in excess of NRC concentrations (tryptophan was not measured). When all crystalline amino acids (including lysine and methionine) were added in excess of NRC requirements to the low-CP diet, feed utilization ($P < 0.05$), but not ADG or ADFI ($P > 0.05$), improved. Yet, the improvement in feed utilization was not sufficiently large to approach that of the chicks fed the control diet (Fancher and Jensen, 1989a). Perhaps 15% was not a sufficiently large increase to overcome a potential deficiency of arginine, threonine, isoleucine, and/or tryptophan. Alternatively, lysine and/or methionine could have been limiting; however, the concentrations of those two amino acids in the plasma were not diminished ($P < 0.05$) in broilers fed the low-CP diet compared with those of broilers fed the high-CP, control diet (Fancher and Jensen, 1989a). Consequently, increasing or decreasing the concentrations of essential amino acids in relation to the NRC-recommended concentrations in low-CP diets by approximately 15% did not explain the reasons for the inferior growth performance.

Dietary electrolyte balance

Maintaining the correct acid–base balance in the body is important from a homeostasis perspective, as even small changes from normal pH may affect enzyme function (Groff and Gropper, 2000). The acid–base balance is maintained through several mechanisms in plasma. Proteins and hemoglobin are important buffers, although the bicarbonate system,

$$CO_2 + H_2O \leftrightarrows H_2CO_3 \leftrightarrows HCO_3^- + H^+ \quad [1]$$

facilitated by the enzyme carbonic anhydrase, is the most important extracellular buffer system (Guyton and Hall, 1996). The bicarbonate system is also used in the renal regulation of acid–base balance, along with the phosphate and ammonia buffer systems. While pH can be regulated though respiration (removal of CO_2 shifts the equilibrium in [1] to the left, removing H^+ from plasma), only little H^+ can be excreted through this system in the urine (Patience, 1990). Instead, H^+ is removed through the phosphate and ammonia buffer systems, the latter of which has implications for protein metabolism. Breakdown of glutamine in the kidneys provides 2 NH_3, with which 2 H^+ can combine and form 2 NH_4^+, which are excreted in the urine. Under normal situations, glutamine—from the diet or synthesized from other (excess dietary) amino acids by transamination of glutamate—is sufficient to meet the need for NH_4^+ synthesis. However, because of the ammonia system's importance in acidotic situations (Guyton and Hall, 1996), skeletal muscle may be broken down to supply sufficient N to excrete the H^+ in the urine (Patience, 1990), consequently lowering N retention and growth performance.

The dietary electrolyte balance (dEB), defined as the dietary content of K + Na – Cl, affects the acid–base balance and, therefore, potentially protein metabolism. The ionic form of Cl, Cl^-, must combine with H^+ to maintain electroneutrality (the H^+ originates from H_2O, [1]). Thus, Cl can be viewed as acidic, as can a relatively low dEB (through either high amounts of Cl

and/or low amounts of K + Na). Crystalline lysine, used in low-CP diets, is generally supplied as the hydrochloride salt, L-lysine·HCl, thereby increasing the acidity of low-CP diets. This effect is exacerbated by the removal of SBM in the low-CP diets, because it (and other plant protein sources) contains relatively high amounts of K (NRC, 1998). Thus, removal of SBM not only results in the addition of Cl (from crystalline lysine), but also in the removal of K, both of which lower the dEB (Patience, 1990), increasing the acidity of the diet. This effect may compromise protein metabolism of animals fed low-CP diets, as the acid–base balance is maintained by the ammonia buffer system, utilizing glutamine from protein (muscle) sources, as mentioned previously. In chickens, an acidic diet does in fact result in increased total urinary N content through an increase in excretion of NH_4^+ (Okumura and Tasaki, 1968). Because the urinary uric acid concentration remained constant in the study by Okumura and Tasaki (1968), chickens may forfeit muscle protein to provide the N in the excreted NH_4^+ to maintain acid–base balance. In contrast, pigs fed a low-dEB (i.e., acidic) diet shift their urinary N excretion from urea to NH_4^+, maintaining total urinary N content (Cai and Zimmerman, 1995; Patience and Chaplin, 1997). As no additional N is secreted by the pigs, the shift enables H^+ excretion while conserving muscle protein, otherwise used to supply the N in NH_4^+. As a result, catabolism of muscle protein to supply glutamine may not have to take place in pigs until severe acidosis is encountered.

Fancher and Jensen (1989a,c), aware of the potential effects of lowering dEB on protein metabolism, formulated low-CP diets for broiler chicks, but kept the K concentrations equal to that found in the high-CP, control diet. However, no beneficial effects of equalizing the dietary K content were found ($P > 0.05$)—the low-CP diets still resulted in an inferior growth performance. In these studies, only K, not Na and Cl (and, therefore, dEB), was kept constant. Hence, the lack of response to the dietary K could have been caused by a potentially lower dEB in the low-CP diets compared with the dEB in the high-CP diet. However, neither dietary

manipulation of the dEB nor additions of antacids and buffers in low-CP broiler diets affected the inferior performance ($P > 0.05$) typical of low-CP diets in trials by Waldroup (2000).

Glutamine, asparagine, and polyamines

There has been little interest in adding the amino acids glutamine and asparagine to low-CP diets, possibly because they have been regarded as nonessential amino acids and can be synthesized in sufficient amounts by the animal from glutamate and aspartate, respectively. Because the content of intact protein is reduced in low-CP diets, the amounts of dietary glutamine and asparagine are also reduced. It is possible that one or both of these amino acids are conditionally essential (Burchman, 1997) and required only in certain situations, one of which might be when animals are fed low-CP diets. If so, one must ask why these requirements cannot be met by de novo synthesis from glutamate and aspartate, respectively, and what roles glutamine and asparagine play in the body. Synthesis of glutamine from glutamate requires NH_3, ATP, and glutamine synthetase, whereas synthesis of asparagine from aspartate requires NH_3 (from glutamine) and ATP and is facilitated by the enzyme asparagine synthetase (Voet and Voet, 1995). One of the substrates (other than glutamate or aspartate) may be in short supply or perhaps the respective synthetase activities are low in pigs fed low-CP diets. In vitro, low glutamine synthetase activities decrease cell proliferation in the IEC-6 rat crypt cell lines (DeMarco et al., 1999) and in Caco-2 cells (Weiss, 1999). Cell proliferation is especially important in the small intestine because the enterocytes (which originate from the crypt cells and are responsible for nutrient absorption) are replaced every two to three days (Johnson and McCormack, 1994).

Glutamine and asparagine have specific functions (other than their incorporation into proteins), which may explain a potential dietary requirement. Glutamine is a major fuel for enterocytes (Windmueller, 1982; Wu et al., 1995); low-CP diets—with reduced amounts of

glutamine—could cause the enterocytes to be energy deficient or to obtain their energy from other, less efficient sources. If so, it is not clear why glutamate cannot replace glutamine as an energy source in the enterocytes as glutamine is converted to glutamate prior to its catabolism in the TCA cycle. Glutamate contributes significantly to the enterocytes' CO_2 production and, therefore, their energy metabolism (Stoll et al., 1999; Reeds et al., 2000). Yet, glutamate has been added to low-CP diets with no effect on growth performance (Pinchasov et al., 1990) and carcass composition (Aletor et al., 2000), suggesting a specific need for glutamine rather than glutamate.

Dietary glutamine plays a role in maintenance of mucosal integrity and growth and has been involved in the recovery from disease, burns, and starvation (Souba et al., 1990; Cynober, 1991; Souba, 1991). In vitro, glutamine stimulates enterocyte protein synthesis (Higashiguchi et al., 1993) and proliferation of enterocytes (Kandil et al., 1995; DeMarco et al., 1999). Moreover, Wu et al. (1996) showed that 1% dietary glutamine (but not 0.6%) prevented atrophy of the jejunal villi of early-weaned pigs. It also significantly improved feed efficiency in the second week after weaning (Wu et al., 1996). In vitro, both glutamine and asparagine stimulate ornithine decarboxylase (ODC) activity in cells of the small intestine (Hayashi, 1989; Kandil et al., 1995; Wang et al., 1996, 1998; Ray et al., 1999). Minami et al. (1985) investigated the effect on ODC activity of feeding individual amino acids to rats and found that glutamate, although structurally similar to glutamine, did not stimulate ODC activity (glutamine, asparagine, and aspartate were not investigated). Similarly, Rinehart et al. (1985) found that, while asparagine stimulated ODC, the acidic aspartate and glutamate did not. In the presence of asparagine, aspartate and glutamate did slightly stimulate ODC activity.

Ornithine decarboxylase facilitates the first and rate-limiting step in the formation of polyamines from ornithine, which, in turn, originates from either arginine or glutamate. Ornithine is decarboxylated to yield putrescine, which is enzymatically (and reversibly) converted

to spermidine and spermine by consecutive enzymatic additions of propylamines originating from S-adenosinemethionine (SAM). Putrescine is degraded by the enzyme diamine oxidase (Johnson and McCormack, 1994). Polyamine synthesis and degradation (and, therefore, the intracellular polyamine concentrations) are highly regulated (Johnson and McCormack, 1994), indicating a likely adverse effect of slightly low and/or slightly high concentrations. Polyamines play important roles in cell differentiation and growth (McCormack and Johnson, 1991; Wang et al., 1991) and are required for normal cell growth (Cohen, 1998). The cellular functions of polyamines are not completely understood; however, they seem to exert their effects through several mechanisms, including effects on the secondary RNA structure, RNA stabilization, translation induction, and RNA degradation (reviewed by Shantz and Pegg [1999] and Igarashi and Kashiwagi [2000]). Although polyamines only have weak interactions with DNA (Igarashi and Kashiwagi, 2000), they may act to prevent oxidative damage to the DNA as a scavenger of free radicals (Ha et al., 1998).

Decreasing the amount of SBM in low-CP diets may also decrease the dietary content of polyamines, which possibly explains the lower growth performance through a scarcity of absorbed, dietary polyamines and/or a concurrent failure to synthesize sufficient amounts of polyamines de novo. Colnago and Jensen (1992) fed low-CP diets to broiler chicks and found significant decreases in gain and feed efficiency when compared with chicks fed control diets. However, no effects of 0.05 or 0.10% supplemental putrescine were found, potentially because the inclusion levels of putrescine were too low. Smith (1990) found that additions of 0.2 and 0.4% putrescine to purified chick diets increased ($P < 0.05$) ADG compared with 0% dietary putrescine. More than 0.6% dietary putrescine decreased ($P < 0.05$) BW gain, however, indicating a potential toxicity of (dietary) polyamines.

Ornithine decarboxylase activity has been found in crypt cells, corroborating the importance of ODC and polyamines in dividing cells. However, the ODC activity is higher in

the nondividing enterocytes than they are in the crypt cells, suggesting additional roles of ODC and perhaps polyamines (Madsen et al., 1996). The research by Madsen et al. (1996) indicated that mitochondrial function in the enterocytes is regulated by polyamine synthesis or ODC activity. The authors found that treatment with difluoromethylornithine (DFMO), an inhibitor of ODC, did not affect CO_2 production from glucose (which can take place in the cytosol through the pentose-phosphate pathway), but decreased the CO_2 production from pyruvate (which takes place in the mitochondria through the pyruvate dehydrogenase complex and the TCA cycle). If pyruvate cannot enter the mitochondria or cannot be metabolized adequately in the mitochondria, pyruvate is instead reduced to lactate (Groff and Gropper, 2000). Madsen et al. (1996) did indeed find that the lactate concentration in DFMO-treated enterocytes were significantly higher than that in control enterocytes. However, ATP concentrations between DFMO-treated cells and control cells were unchanged, which also was noted in an experiment by Yang et al. (1999). The similar ATP concentrations indicate that the cells may obtain energy (ATP) through other pathways (e.g., anaerobic glycolysis), which may be less efficient than the 'normal' pathways (i.e., the TCA cycle and the electron transport chain). It was also shown by Madsen et al. (1996) that DFMO had a negative effect on the morphology of the mitochondria in which the TCA cycle reactions take place. Hence, ODC (and/or polyamines) may be involved in maintaining mitochondrial integrity (and, therefore, function) in the absorptive cells of the small intestine (Madsen et al., 1996). ODC may be involved in pyruvate entry into mitochondria or in the regulation of TCA-cycle activity (Madsen et al., 1996), which lends a basis for the above-mentioned suggestion that enterocyte energy metabolism may be inefficient when the ODC activity is low, caused by the ingestion of glutamine/asparagine-deficient, low-CP diets. It may also help explain the lack of effect of dietary polyamines observed by Colnago and Jensen (1992) as preformed (i.e., dietary) polyamines may not be needed, but rather a high activity of ODC and/or polyamines synthesized de novo. In addition, semipurified diets, fortified with crystalline

amino acids (but without glutamine and asparagine), have been shown to result in decreased intestinal ODC activity when compared with isonitrogenous, intact-protein diets (Guihot et al., 1997; Yang et al., 1999). Dietary glutamine and/or asparagine may function to stimulate ODC activity, which, in turn, is necessary for optimal energy metabolism in the cells of the small intestine.

Although the concentrations of ATP are unaffected by DFMO treatment (Madsen et al., 1996; Yang et al., 1999), the stability of ATP may be affected by the presence of polyamines because spermine forms a 1:2 complex with ATP (Meksuriyen et al., 1998). Binding of spermine to ATP may also facilitate reactions involving ATP, such as ATPases and protein kinases (Meksuriyen et al., 1998). With diminished ODC activity and/or polyamine concentrations, as may be the case in enterocytes after ingestion of low-CP diets, cellular reactions involving ATP may be compromised.

Intact protein, peptides, and free amino acids

The majority of dietary protein from intact protein is absorbed into the enterocytes of the small intestine as di- and tripeptides. Only relatively little is absorbed as free amino acids (Zaloga, 1990; Ganapathy et al., 1994). The absorption of N from peptides is influenced by the peptide chain length when peptides containing under five amino acid residues are fed. Grimble et al. (1987) perfused various mixtures of peptides containing between two and five amino acid residues derived from egg-white protein into the duodenum of adult humans. Perfusion of a di- and tripeptide mix resulted in higher ($P < 0.05$) N and amino acid absorption than did a mix of tri, tetra-, and pentapeptides. Hence, peptides, longer than tripeptides, are not readily absorbed and have to be hydrolyzed by (brush border) enzymes before absorption in large amounts can occur (Grimble et al., 1987; Matthews, 1991).

Free amino acids present in the lumen of the small intestine are actively transported into the enterocytes by various Na^+-dependent and Na^+-independent (contingent on the physico–chemical properties of the amino acid) mechanisms. Di- and tripeptides from the enzymatic hydrolysis of the ingested (intact) protein are actively transported from the intestinal lumen into the enterocytes, albeit by a different mechanism than that of the free amino acids, requiring H^+ rather than Na^+ (Ganapathy et al., 1994). Whereas one Na^+ is required for absorption of (most) free amino acids (Ganapathy et al., 1994), it is believed that one H^+ is exchanged for the absorption of one basic or neutral peptide by the small-intestinal peptide transporter PepT1, while acidic peptides require cotransport with two H^+ (Steel et al., 1997). Establishment of a Na^+ gradient, utilized to drive the absorption of free amino acids, costs ⅓ ATP per Na^+ through Na^+/K^+ ATPase. The Na^+/K^+ ATPase also plays a role in the absorption of peptides, although more indirectly: A Na^+/H^+ exchanger is responsible for the lower pH (corresponding to a higher H^+ concentration) immediately outside the enterocyte, producing the H^+ gradient that drives the peptide absorption. The stoichiometry of the Na^+/H^+ exchanger is 1:1 (Ganapathy et al., 1994), meaning that the cost of expelling the 1 H^+ required for absorption of one (basic or neutral) peptide is 1 Na^+. Expelling 1 Na^+, in turn, costs ⅓ ATP through the Na^+/K^+ ATPase. It follows that the cost of absorbing acidic peptides, requiring 2 H^+, is ⅔ ATP. Hence, absorption of amino acids in the form of peptides, rather than free amino acids, is energetically favorable: Absorption of one *peptide-bound* amino acid costs between ⅓ and ⅔ ATP per amino acid depending on the peptide length, assuming that 1 H^+ (and therefore 1 Na^+) is required for absorption of the peptide. In contrast, absorption of one *free* amino acid costs ⅓ ATP. Potentially, low-CP diets—with their inherently high amounts of free amino acids—may not be utilized as well as diets based on intact protein because of the inefficiencies associated with absorption of a large proportion of the dietary protein.

Batterham (1974) raised the concern that the utilization of crystalline amino acids in pigs fed once a day would decrease if a relatively high amount of one crystalline amino acid (lysine) was fed together with intact protein. This idea is based on the different absorption rates of dietary crystalline amino acids and dietary intact protein, of which the latter cannot be absorbed without prior (time-consuming) digestion. Therefore, the intestinal supply of absorbable amino acids at a given time may be imbalanced and dietary crystalline amino acids will appear sooner in the blood than (the other) amino acids from intact protein (Buraczewska et al., 1980; Rérat et al. 1984; Dangin et al., 2001). As a result, there will be an imbalance in the plasma amino acid profile, potentially leading to oxidation of the "surplus" amino acids. By the time amino acids from the intact protein appear in the plasma, (at least some of) the amino acids supplied from crystalline sources are missing, decreasing the capacity for protein synthesis and, consequently, the biological value (BV) of the dietary protein. This latter concept was demonstrated in the classical amino acid deletion studies by Rose and his students (e.g., Rose et al., 1950, 1955).

Di- and tripeptides (either specific or mixes from partial enzymatic hydrolysates of intact protein) are absorbed from the intestinal lumen at a faster rate than the corresponding free amino acids. Burston et al. (1980) studied the kinetics of L-lysine and L-lysyl–L-lysine absorption in everted hamster intestine in vitro. They found that at concentrations above 10 mM, the dipeptide was absorbed faster (higher V_{Max}) than the free amino acid from equimolar lysine concentrations. The opposite was found at lysine concentrations below 10 mM. The total lysine uptake was higher if absorbed from lysyl–lysine than from free lysine (Burston et al., 1980), suggesting a disadvantage of feeding diets high in crystalline amino acids. Steinhardt and Adibi (1986) found that at concentrations of 6 mM absorption was greater ($P < 0.05$) for dipeptides than for the corresponding free amino acids. Generally, Steinhardt and Adibi (1986) found that N absorption was greater from peptides than from free amino acids in humans.

Similarly, Hara et al. (1984) showed that amino acid N absorption was higher ($P < 0.05$) from di- and tripeptides than from free amino acids in conscious, unrestrained rats fitted with duodenal and portal catheters for infusion and blood sampling. Infusions consisted of either an enzymatic hydrolysate of egg-white protein or a mixture of free, crystalline amino acids, both of which had a similar amino acid balance. Peak concentrations in the portal blood of essential amino acids were reached 5 to 15 minutes after infusions of di- and tripeptides as compared with 10 to 30 minutes after infusions of free amino acids. This difference supports research performed by Rérat et al. (1984, 1988, 1992) in which amino acid N absorption was greater, faster, and more homogeneous when peptides, rather than crystalline amino acids, were administered to pigs. Hence, while dietary free amino acids are absorbed faster than dietary intact protein, the intact protein, once it has been hydrolyzed to di- and tripeptides, is absorbed faster than the free amino acids.

The difference in amino acid absorption rates between dietary free and peptide-bound amino acids becomes especially important in meal-fed animals (Batterham, 1974; Batterham and O'Neill, 1978). However, most growing animals have free access to feed and tend to eat throughout the day (Bigelow and Houpt, 1988; Hyun et al., 1997, 1998). It follows that the stomach and small intestine should contain digesta at all times, which will minimize potential effects of different absorption rates of the dietary amino acids. Frequent feeding does eliminate some of the differences in appearance of amino acids in plasma (Buraczewska et al., 1980). However, Metges et al. (2000) showed that, even with hourly meals, changes in plasma amino acid concentrations were much more pronounced if the dietary amino acids came from free (crystalline) amino acids instead of from intact protein. Moreover, pigs and poultry with free access to feed tend to eat several large meals per day, with the majority of the daily feed intake concentrated in the mornings (Fraser and Broom, 1990; Hyun et al., 1997). Furthermore, the number of meals per day decreases with age (Bigelow and Houpt, 1988; Hyun et al., 1997).

Although the feed intake of animals with ad libitum access to feed is spread out over the course of the day, it is possible that a diurnal absorption pattern of amino acids from the small intestine exists, with at least some effect on the appearance of dietary amino acids in the plasma. Feeding a large proportion of the dietary protein in the form of free, crystalline amino acids rather than as intact protein (or peptides) may have negative effects on amino acid absorption rates and their appearance in the portal blood.

Bioavailability and biological value

Crystalline, or synthetic, amino acids are assumed to be 100% absorbed from the small intestine and 100% available for protein synthesis (Izquierdo et al., 1988; Radke and Lewis, 1992). The true digestibility of crystalline amino acids was 100% (or, rather, not significantly different from 100%) in ileal-cannulated pigs and cecectomized cockerels (Izquierdo et al., 1988; Chung and Baker, 1992). Conceivably, all dietary crystalline amino acids are available for protein synthesis (disregarding the catabolism of all amino acids in the enterocytes described by Stoll et al. [1998]) in the animal and the crystalline amino acids therefore are of high BV. Biological value is defined as the ratio of retained to absorbed N and a relatively higher BV indicates that a higher proportion of absorbed protein is used for protein synthesis. For crystalline amino acids to have a high BV, all amino acids must be present (available) at the place of protein synthesis at the same time. This condition can be achieved with diets containing all the essential (and at least some of the nonessential) amino acids in crystalline form. If just one of the (essential) amino acids is supplied in free (crystalline) form, it may appear in the enterocyte and portal blood (and subsequently at the site of protein synthesis) sooner than the other amino acids supplied from intact protein because of the differences in the digestion/absorption rate between the two forms of protein. This potential problem of low-CP diets is amplified in meal-fed animals (discussed by Batterham [1974]), but may occur in animals with free access to (low-CP) diets

(discussed in the previous section, *Intact protein, peptides, and free amino acids*). Consequently, the protein in low-CP diets may be of low BV despite the "100% availability" of crystalline amino acids. This may, in turn, lead to an increased catabolism of the amino acids supplied in crystalline form. If all amino acids are supplied in crystalline (free) form, digestion/absorption times may be similar—as will growth performance and N retention. The latter was demonstrated in experiments by Chung and Baker (1991) in which piglets fed diets with all amino acids derived from either intact protein (i.e., corn, SBM, and whey) or crystalline amino acid had similar ($P > 0.05$) growth performance and N retention.

Maillard reactions between the amino group of amino acids and the carbonyl group of reducing sugars in the diet will decrease the availability of amino acids (and the sugars). Intact protein is less susceptible to Maillard reactions than are free amino acids because relatively few amino groups are available for the reaction. It follows that low-CP diets, with their high amounts of crystalline, free amino acids are especially susceptible to Maillard reactions, potentially lowering the quality and availability of the dietary protein. Mavromichalis and Baker (2000) found that, although crystalline lysine was 100% bioavailable in pig diets, the bioavailability decreased ($P < 0.05$) to 90% after one week of storage in a hot and humid nursery facility. This decrease implies that the protein quality and amino acid bioavailability of diets containing crystalline amino acids decreases rapidly during storage. Although low-CP diets normally contain few reducing sugars, they do contain large amounts of free (crystalline) amino acids, making a large proportion of the protein in low-CP diets susceptible to degradation when stored in warm, humid conditions. Therefore, the inferior growth performance observed with low-CP diets may be because of a decrease in protein bioavailability after as little as one week in storage.

OBJECTIVE OF THE DISSERTATION RESEARCH

Growth performance and carcass quality of broiler chicks fed low-CP diets have been shown in several experiments (e.g., Fancher and Jensen, 1989a,b,c; Pinchasov et al., 1990; Ferguson et al., 1998; Aletor et al., 2000) to be inferior to that of chicks fed a standard, high-CP diet. These adverse effects also occur in growing pigs (e.g., Kerr et al., 1995; Tuitoek et al., 1997; Knowles et al., 1998) even though the low-CP diets meet established amino acid requirements and ratios of essential-to-nonessential amino acids. Several studies have been performed to investigate potential reasons for the low performance. They include modifying the dietary NE concentrations (Knowles et al., 1998; Leeson et al., 2000), additions of several crystalline nonessential amino acids (Edmonds et al., 1985; Deschepper and De Groote, 1995; Aletor et al., 2000), decreasing the dietary concentrations of essential amino acids by as much as 13% in relation to recommended concentrations (Pinchasov et al., 1990), increasing the dietary concentrations of essential amino acids by up to 15% in relation to recommended concentrations (Fancher and Jensen, 1989a,c), and manipulating the dietary acid–base balance (Fancher and Jensen, 1989a,c; Waldroup, 2000). Yet, the reason (or reasons) for the inferior performance has yet to be determined. Therefore, broiler chicks and pigs cannot be fed diets with CP contents less than three to four percentage points below NRC (1994, 1998) recommended concentrations without forfeiting growth performance and/or carcass quality (Lewis, 2001).

Potential reasons suggested for the inferior performance of broilers and pigs fed low-CP diets include an increased NE content of low-CP diets. Low-CP diets are inherently higher in NE, because corn, which replaces SBM in the low-CP diet, contains considerably more NE than does SBM (De Groote, 1974; NRC, 1998). Other potential reasons include growth factors present in SBM, but not in corn and crystalline amino acids (Cook, 1998; Greiner, 1998); changes in dietary electrolyte and acid–base balance (Patience, 1990; Patience and Chaplin, 1997); a failure

to meet the amino acid requirements (Lewis, 2001); or a decreased bioavailability of crystalline amino acids after storage of the diets (Mavromichalis and Baker, 2000).

Dietary intact protein is absorbed in the small intestine as a combination of free amino acids and di- and tripeptides with the majority absorbed in peptide form (Matthews, 1991; Ganapathy et al., 1994; Groff and Gropper, 2000). Correspondingly, Rérat et al. (1984, 1988, 1992) have shown that absorption of amino acids from peptides is greater, faster, and more homogenous than amino acid absorption from free (crystalline) amino acids. Because crystalline amino acids are considered 100% bioavailable (Izquierdo et al., 1988; Chung and Baker, 1992), the differences in absorption mechanisms between amino acids from intact protein and crystalline sources may lead to differences in their metabolism. In turn, these differences may be responsible for the inferior utilization of low-CP diets, in which a large proportion of the protein originates from free amino acids. In Experiment 1, this concept was investigated through two diets of similar composition: One diet supplied amino acids from intact protein, whereas another diet supplied approximately half its protein from crystalline (free) amino acids. The true digestible contents of all essential and all nonessential amino acids were similar between the two diets, which only differed in the molecular form of the amino acids.

Glutamine and asparagine, as well as their precursors, glutamate and aspartate, are inherently low in low-CP diets because the amount of their main source, SBM, is decreased in the diets. All four amino acids—especially glutamate and glutamine—are important energy sources for enterocytes (Windmueller and Spaeth, 1976; Windmueller, 1982; Wu et al., 1995; Reeds et al., 2000). Decreased dietary amounts of these amino acids may affect energy metabolism in the enterocytes. Furthermore, glutamine and asparagine stimulate ODC activity in the enterocytes (Wang et al., 1996, 1998; Ray et al., 1999), stimulate enterocyte proliferation (Kandil et al., 1995; DeMarco et al., 1999), stimulate enterocyte protein synthesis (Higashiguchi et al., 1993), increase jejunal villus height (Salloum et al., 1989; Wu et al., 1996), and improve feed

utilization in early-weaned pigs (Wu et al., 1996). The diets in Experiment 1 did not contain any crystalline glutamine and asparagine because the amounts of those in corn and SBM are unknown caused by difficulties in their analysis (Nissen, 1992). Experiment 2 was thus designed to investigate if dietary glutamine or asparagine were limiting performance of chicks fed low-CP diets.

All crystalline amino acids are assumed to be 100% true digestible (Izquierdo et al., 1988; Chung and Baker, 1992); all crystalline amino acid are presumably absorbed fully into the enterocytes. However, if free (crystalline) amino acids are catabolized to a larger extend in the enterocytes than peptides (originating from intact protein), crystalline amino acids may not be 100% available for lean growth in spite of their 100% digestibility. Stoll et al. (1998) showed that approximately 30% of dietary essential amino acids are catabolized in the enterocytes. As suggested above, it is possible that free amino are metabolized differently in the enterocytes than peptides—potentially decreasing their bioavailability. Similarly, the bioavailability of crystalline amino acids in low-CP diets may decrease after as little as 1 wk of storage (Mavromichalis and Baker, 2000), and the chicks' capacity for protein deposition may therefore also decrease. In both scenarios, increasing the dietary concentrations of crystalline amino acids may help overcome this potentially low availability of crystalline amino acids. A third experiment was designed to investigate if increasing the concentrations of crystalline amino acids by up to 45% in low-CP diets would restore performance to that of chicks fed a high-CP control diet.

The dietary CP requirements for 0- to 3-wk-old broiler chicks is 23% CP (NRC, 1994), of which the required essential amino acids (proline and serine not included) make up 40%, corresponding to 9.2 percentage points. The remaining CP should come from nonessential amino acids and/or essential amino acids in excess of the requirements. Bedford and Summers (1985) found that in broiler chicks, 7 to 21 d of age, the optimal ratio between the dietary concentrations

of essential and nonessential amino acids was 55:45 for growth performance and 65:35 for N retention. Low-CP diets contain fewer essential amino acids in excess of the requirements, as well as fewer nonessential amino acids. In this way, it is possible that low-CP diets are deficient in nonspecific N (i.e., nonessential amino acids). If so, chicks fed a low-CP diet should respond favorably to dietary additions of nonessential amino acids. Fancher and Jensen (1989a,b,c) observed improvements ($P < 0.05$), but neither Deschepper and De Groote (1995) nor Aletor et al. (2000) observed significant improvements in carcass quality of broiler chicks after nonessential amino acids were added to low-CP diets. Growth performance, however, was improved in the study by Aletor et al. (2000), but not in those by Fancher and Jensen (1989a,b,c) or Pinchasov et al. (1990). Experiment 4 was designed to investigate if the inferior growth performance of chicks fed low-CP diets in Experiments 1, 2, and 3 was because of a deficiency of nonspecific N. The low-CP diets in Experiment 4 were formulated to contain increasing concentrations of crystalline nonessential amino acids to investigate if the addition of nonspecific N would improve growth performance and carcass quality.

The objective of the dissertation research was to investigate potential reasons why low-CP diets result in inferior performance and, in so doing, attempt to improve the growth performance. The objective was approached through a series of four growth performance and N retention experiments with broiler chicks. The experiments were designed to investigate effects of 1) the molecular form of the protein, 2) the influence of dietary glutamine and asparagine, 3) the concentrations of dietary crystalline essential amino acids, and 4) the concentrations of dietary crystalline nonessential amino acids. All four experiments used a similar experimental design, differing in the number and type of diets fed.

MATERIALS AND METHODS

Material and methods common to all four experiments

Day-old male broiler chicks (Table 1) were purchased from a commercial hatchery (Welp Inc., Bancroft, IA, 50517), placed in floor pens (1.3 × 1.3 m) containing pine shavings and given free access to water and a common diet (23% CP) until allotment to dietary treatments on d 7 posthatching. Lights were on continuously for the first 10 d posthatching, after which a 17-h-on, 7-h-off lighting schedule was maintained for the duration of the trials. The room temperature (measured approximately 1.5 m above the floor) was maintained at approximately 26°C for the first 2 d, after which it was gradually lowered to 21°C. Each pen was furnished with a heat lamp to keep floor-height pen temperatures at approximately 35°C for the first 2 d, after which the lamps were gradually raised and eventually removed between 7 and 14 d posthatching. On d 7 posthatching all chicks were weighed and six chicks with a representative BW were selected, fasted overnight (with free access to water), euthanized by cervical dislocation, and frozen at −20°C for later determination of the baseline whole-body dry matter (DM) and N contents.

In all trials, a completely randomized design was used with four to six dietary treatments and six replicate pens (experimental units) containing 10 chicks (Table 1). Chicks were allotted on the basis of BW on d 7 posthatching to pens (1.3 × 1.3 m), which were randomly allotted to dietary treatments. All chicks (except the six baseline chicks) were given free access to their

Table 1. Broiler chicks used in the four experiments.

Experiment	Broiler chick strain	Dietary treatments	Replications[1]	Total number of chicks[2]	Initial body weight,[3] g
1	Petersen × Hubbard	4	6	246	120.7 ± 0.7
2	Petersen × Hubbard	4	6	246	128.3 ± 0.2
3	Petersen × Hubbard	5	6	306	146.4 ± 0.1
4	Hubbard × Hubbard	6	6	366	122.9 ± 0.4

[1]Each experiment had six replicate pens, each containing 10 chicks, per treatment.
[2]Number of chicks used in each experiment (includes six baseline chicks).
[3]Body weight of individual chicks at allotment on d 7 posthatching (mean ± standard error).

respective treatment diets and water for the subsequent 2 wk. Feed disappearance and BW were measured on d 14 and 21 posthatching. After the chicks were weighed on d 21, they were fasted overnight (with free access to water), and re-weighed on d 22. Subsequently, two chicks from each pen (with a BW close to the pen mean) were selected, euthanized by cervical dislocation, and frozen at –20°C for later determination of the whole-body DM and N contents. The mean whole-body composition of the two chicks per pen was used to calculate the mean whole-body composition of the 10 chicks in each pen. All procedures relating to the use of live animals were approved by the University Committee on Animal Care before the onset of each experiment.

Diet formulation

When formulating the diets, all crystalline amino acids were assumed to be 100% true digestible (Izquierdo et al., 1988; Chung and Baker, 1992). However, the true digestibilities of amino acids from corn and SBM range from 81 to 94% with an average of about 88% true digestibility (NRC, 1994). This difference makes direct comparisons of the dietary amino acid contents difficult between diets containing high amounts of crystalline amino acids and diets based on intact protein. Hence, in regard to amino acids, dietary formulations in all experiments were based on a true digestible basis. The NRC (1994) does not list requirements for true digestible amino acids, only for total amino acids. Therefore, the requirement for true digestible amino acids was estimated to be 89 or 90% (depending on the experiment) of the total amino acid requirement, based on the 88% average digestibility of amino acids in corn and SBM. Dietary concentrations of amino acids in Experiment 1 were those listed by the NRC (1994), without adjustment for true digestibility (i.e., the requirement for total amino acids was used in Experiment 1). Nitrogen-corrected ME (ME_n) values were used for all ingredients to formulate isoenergetic diets containing 3200 kcal ME_n/kg.

L-Lysine·HCl, DL-methionine, L-threonine, and L-tryptophan used in the diets were feed grade. All other crystalline amino acids (in their L-form) as well as K_2CO_3 and Na_2CO_3 were reagent grade (minimum 98% purity) and purchased from Sigma-Aldrich Chemical (St. Louis, MO, 63178).

Whole-body homogenizing and sampling

Whole bodies were homogenized and sampled according to procedures described by Barker and Sell (1994) modified from Sibbald and Wolynetz (1984): The whole body of individual chicks were combined with distilled water (1 × BW) in glass beakers, autoclaved for 8 h, and allowed to cool overnight in the autoclave. Weight loss during this process was assumed to be water, which was replaced and the birds subsequently blended in a Waring blender (Model 38BL19, Waring Products Division, New Hartford, CT, 06057) for 2 min after addition of distilled water (2 × BW). Duplicate aliquots of 90 to 100 g were dried in plastic weigh boats at 50°C, ground with a mortar and pestle, and stored in Whirl Pack bags at room temperature for later Kjeldahl N analysis.

Whole-body composition, nitrogen retention, nitrogen utilization, and nitrogen excretion

Whole-body DM was calculated from the dry weight of the ground chicks in addition to records of additions and losses of water (Barker and Sell, 1994). Percent whole-body water was subsequently calculated by difference. Kjeldahl N was determined according to AOAC (1984) using the micro-Kjeldahl method on a Kjeltech 1028 distilling unit (U.S. Tecator Inc., Herndon, VA, 22070). Whole-body CP was calculated as Kjeldahl N × 6.25. Body fat was estimated from the percent whole-body water according to Velu et al. (1972). Although the prediction equation for body fat composition was developed for chicks 21 d of age, the equation was used to predict body fat composition for the baseline chicks (7 d of age). However, this was not done in Experiments 2 and 4, because the water content of the baseline chicks was more than 74%

(Velu et al., 1972). Therefore, fat retention was calculated only in Experiments 1 and 3 to be the difference between the calculated whole-body fat content at the end of trial and the calculated baseline whole-body fat content.

N retention was calculated by computing the difference between the whole-body N content at the end of the trial (after 2 wk on test) and the whole-body baseline N content. Efficiency of N use was calculated as N retained divided by the N intake. Protein efficiency ratio (PER) was calculated using CP intake (calculated from the ADFI) and the analyzed dietary CP content as shown in [2].

$$\text{Protein efficiency ratio} = \text{Grams of gain in 2 wk} \div \text{Grams of CP consumed in 2 wk} \quad [2]$$

Moreover, apparent N excretion was calculated as the difference between N intake and N retention. The (apparent) N excretion as a percentage of N intake was calculated to account for potential differences in N intakes between the dietary treatments. Analyzed N values were used in all calculations.

Mortality

Weight gain and feed intake of dead or culled chicks were not used in the calculations of the pen means for ADG, ADFI, and feed utilization (gain-to-feed ratio, G:F). On the day of death, feeders were weighed in the affected pen and the feed intake calculated for individual chicks since the previous weigh day. The feed intake attributed to the dead chick was subsequently subtracted from the pen feed intake on the following weigh day.

Experiment 1

Diets

In Experiment 1, it was decided to formulate the diets using table values for amino acid composition of the ingredients rather than analyzed values. This decision was based on the large analytical variability among laboratories (Cromwell et al., 1999), meaning that the NRC table value for a given amino acid would be within the range of amino acid values originating from analyses at (several) different laboratories. As a result, the feed ingredient composition (including amino acids and other nutrients) of all feed ingredients used in Experiment 1 were table values published by the NRC (1994) and Degussa (1995).

A common corn–SBM diet (similar to Diet 1A) was fed from d 1 to 7 posthatching. The control diet, Diet 1A, was formulated as a 23% CP 'standard' or 'practical' diet, meeting or exceeding the NRC (1994) requirements for total amino acids using corn and SBM (Table 2). Using true digestibility values from the NRC (1994) and, if not available from NRC (1994), apparent digestibilities published by Heartland Lysine (1998) for the amino acids in corn and SBM, the amounts of true digestible amino acids in Diet 1A were calculated. These amounts were matched in Diets 1B and 1C using corn, SBM and crystalline amino acids. The true digestibility of crystalline amino acids was assumed to be 100% (Izquierdo et al., 1988; Chung and Baker, 1992). Cornstarch was added at the expense of corn and SBM in Diets 1B, and 1C. When appropriate, crystalline tryptophan was added as Tryptosine™, a blend of L-tryptophan (70%) and L-lysine·HCl (30%). The contents of glutamine and asparagine in corn and SBM were unknown so the two amino acids were added as L-glutamate and L-aspartate, respectively. Diet 1D was formulated as a low-CP diet to serve as a negative control diet. All diets contained identical corn:SBM ratios to minimize interactions between the two and were formulated to meet or exceed NRC (1994) requirements for essential amino acids, ME_n, minerals, and vitamins

Table 2. Composition of diets in Experiment 1 (as-fed basis).

Ingredient	Diet 1A Intact protein (positive control) %	Diet 1B Crystalline essential and nonessential amino acids %	Diet 1C Crystalline essential amino acids %	Diet 1D Low-CP (negative control) %
Corn	51.18	26.10	26.10	35.35
Soybean meal (48% CP)	38.25	19.50	19.50	26.40
Cornstarch	–	34.59	40.84	27.68
Soybean oil	6.50	2.40	1.15	3.50
Dicalcium phosphate	1.73	2.05	2.05	1.94
Limestone	1.33	1.29	1.29	1.31
Solka-floc™	–	1.29	1.29	0.81
K_2CO_3	–	1.09	1.09	0.69
NaCl (iodized)	0.25	0.02	0.02	0.18
Na_2CO_3	–	0.22	0.22	0.07
Mineral premix[1]	0.30	0.30	0.30	0.30
Vitamin premix[2]	0.30	0.30	0.30	0.30
L-Alanine	–	0.52	–	–
L-Arginine	–	0.73	0.73	0.16
L-Aspartate	–	1.17	–	–
L-Cysteine	–	0.16	–	–
L-Glutamate	–	1.88	–	–
Glycine	–	0.44	0.15	–
L-Histidine	–	0.28	0.28	–
L-Isoleucine	–	0.46	0.46	0.12
L-Leucine	–	0.92	0.92	–
L-Lysine·HCl	–	0.07	0.07	0.25
DL-Methionine	0.16	0.33	0.48	0.40
L-Phenylalanine	–	0.52	0.93	–
L-Proline	–	0.60	–	–
L-Serine	–	0.52	–	–
L-Threonine	–	0.39	0.39	0.19
L-Tyrosine	–	0.42	–	–
Tryptosine™	–	0.95	0.95	–
L-Valine	–	0.49	0.49	0.15
Total	100.00	100.00	100.00	100.00

CP, crude protein.

[1]Supplied per kg of diet: Manganese, 70 mg; zink, 90 mg; iron (ferrous sulfate), 60 mg; copper, 12 mg; selenium (sodium selenite), 0.15 mg; sodium chloride, 2.5 g.

[2]Supplied per kg of diet: Vitamin A (retinyl acetate), 8065 IU; cholecalciferol, 1580 IU; vitamin E (DL-α-tocopheryl acetate), 15 IU; vitamin B_{12}, 16 µg; vitamin K (menadione sodium bisulfite), 4 mg; riboflavin, 7.8 mg; pantothenic acid, 12.8 mg; niacin, 75 mg; choline, 509 mg; folic acid, 1.62 mg; biotin, 270 µg.

(Table 3). The CP contents of the diets were determined using the micro-Kjeldahl method on a Kjeltech 1028 distilling unit (U.S. Tecator Inc., Herndon, VA, 22070). The dietary amino acid contents were analyzed using ion-exchange chromatography and detected by flourometry after post-column derivatization with o-phthalaldehyde on an automated amino acid analyzer (Shimadzu HPLC LC-6A Series; Tokyo, Japan).

Based on calculated values, all four experimental diets met or exceeded NRC (1994) requirements for ME_n, essential amino acids, minerals, and vitamins. The dEB (calculated as Na + K – Cl), crude fiber, and ME_n contents were held constant in all diets to minimize effects of the dietary acid–base balance, fiber, and energy, respectively (Table 3). The CP contents of the ingredients and diets were determined using the micro-Kjeldahl method on a Kjeltech 1028 distilling unit (U.S. Tecator Inc., Herndon, VA, 22070).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) procedures appropriate for a completely randomized design (Morris, 1999) using the 'Fit Model' command in JMP 4.0.3 (SAS Inst. Inc., Cary, NC). Treatment means were separated by Fischer's protected least significant difference (LSD) using StatView 5.0.1 (SAS Inst. Inc., Cary, NC). P-values of 0.05 or less were considered significant.

Experiment 2

Because the results of Experiment 1 showed differences in growth performance between Diets 1A and 1B, it was thought that dietary glutamine and/or asparagine, which were not added to Diet 1B, may have had an effect on performance. Experiment 2 was therefore designed to investigate the effects of dietary glutamine and asparagine on growth performance, N retention, and whole-body composition.

Table 3. Chemical composition of diets in Experiment 1 (as-fed basis).

Item	Diet				Requirement[1]
	1A	1B	1C	1D	
Analyzed values					
Crude protein (total), %	24.59	22.32	18.76	18.85	23.00
Calculated values					
Crude protein (total), %	22.99	20.55	16.99	17.00	23.00
ME_n, Mcal/kg	3.20	3.20	3.20	3.20	3.20
Ether extract, %	8.83	3.66	2.43	5.17	–
Linoleic acid, %	4.37	1.77	1.14	2.52	1.00
Crude fiber, %	2.62	2.62	2.62	2.62	–
Calcium, %	1.00	1.00	1.00	1.00	1.00
Phosphorus (available), %	0.45	0.45	0.45	0.48	0.45
Potassium, %	0.91	0.91	0.91	0.91	0.30
Sodium, %	0.20	0.20	0.20	0.20	0.20
Chloride, %	0.33	0.33	0.33	0.33	0.20
Dietary electrolyte balance,[2] %	0.79	0.79	0.79	0.79	–
Corn:soybean meal ratio	1.34	1.34	1.34	1.34	–
CP_{EAA}:CP_{total} ratio,[3] %	51.8	57.8	69.0	55.3	–
True digestible amino acids[4]					
Alanine, %	1.03	1.03	0.52	0.71	–
Arginine, %	1.46	1.46	1.46	1.17	–
Aspartate + asparagine, %	2.33	2.33	1.19	1.61	–
Cysteine, %	0.32	0.32	0.16	0.22	–
Glutamate + glutamine, %	3.76	3.76	1.92	2.59	–
Glycine, %	0.88	0.88	0.60	0.61	–
Glycine + serine, %	1.93	1.93	1.13	1.34	–
Histidine, %	0.56	0.56	0.56	0.39	–
Isoleucine, %	0.91	0.91	0.91	0.74	–
Leucine, %	1.85	1.85	1.85	1.28	–
Lysine, %	1.18	1.18	1.18	1.01	–
Methionine, %	0.49	0.49	0.64	0.62	–
Methionine + cysteine, %	0.80	0.81	0.80	0.84	–
Phenylalanine, %	1.04	1.04	1.44	0.72	–
Phenylalanine + tyrosine, %	1.87	1.88	1.87	1.29	–
Proline, %	1.20	1.21	0.61	0.83	–
Serine, %	1.05	1.05	0.54	0.73	–
Threonine, %	0.78	0.78	0.78	0.73	–
Tryptophan, %	0.28	0.28	0.28	0.20	–
Tyrosine, %	0.84	0.84	0.43	0.58	–
Valine, %	0.98	0.98	0.98	0.83	–

ME_n, nitrogen-corrected metabolizable energy.

[1]Source: NRC (1994).

[2]K + Na – Cl.

[3]Percent of crude protein originating from essential amino acids (proline was considered a nonessential amino acid).

[4]Calculated using digestibility coefficients from NRC (1994) and Heartland Lysine (1998).

Table 4. Analyzed amino acid and crude protein concentrations of the corn and soybean meal used in Experiment 2 (as-fed basis).

Amino acid	Corn		Soybean meal	
	Total[1] %	True digestible[2] %	Total[1] %	True digestible[2] %
Arginine	0.35	0.31	3.46	3.18
Cysteine	0.17	0.14	0.78	0.64
Glycine[3]	0.29	0.25	2.02	1.82
Histidine	0.21	0.20	1.31	1.15
Isoleucine	0.24	0.21	2.24	2.08
Leucine	0.77	0.72	3.66	3.37
Lysine	0.26	0.21	3.13	2.85
Methionine	0.16	0.15	0.67	0.62
Phenylalanine	0.32	0.29	2.42	2.30
Proline[3]	0.56	0.49	2.25	2.02
Serine[3]	0.26	0.23	1.94	1.75
Threonine	0.23	0.19	1.78	1.57
Tryptophan	0.05	0.04	0.76	0.67
Tyrosine	0.20	0.18	1.64	1.48
Valine	0.35	0.31	2.48	2.26
Crude protein[4]	7.72	–	48.44	–

[1]Total amino acid content.
[2]Calculated from true digestibility coefficients (NRC, 1994) and used in formulation of Diets 2A through 2D.
[3]The true digestibility coefficient was calculated as the mean of all other amino acids' true digestibility coefficients.
[4]Kjeldahl N × 6.25.

Diets

Corn and SBM were analyzed for total amino acid contents at a commercial laboratory (Experiment Station Chemical Laboratories, Columbia, MO, 65211) before diet formulation, after which the contents of true digestible amino acids were calculated (Table 4) from digestibility coefficients listed by the NRC (1994). These figures were subsequently used for formulation of the diets. A common diet (similar to Diet 2A; Table 5), formulated with corn and SBM to meet or exceed NRC (1994) requirements, was fed to all the chicks from d 1 to 7 posthatching. From d 7 to 21 posthatching, the chicks were fed one of the four treatment diets, which were formulated to meet or exceed the NRC (1994) requirements for energy, minerals, and vitamins. The positive control diet (Diet 2A) was formulated using corn, SBM, and crystalline DL-methionine to meet the NRC (1994) recommendations of 23% CP and 3200 kcal ME_n per kg diet. A negative control

diet (Diet 2B) was formulated to contain 18% CP using corn, SBM, and crystalline amino acids to meet the true digestible amino acid requirement (i.e., 90% of total amino acids listed by the NRC [1994]). Diets 2C and 2D were formulated as Diet 2B and contained 1% crystalline L-glutamine or L-asparagine, respectively, replacing triammonium citrate (TAC; Table 5). A concentration of 1% supplemental glutamine was chosen because Wu et al. (1996) found responses to 1% glutamine in early-weaned pigs. Triammonium citrate, rather than a nonessential amino acid, was used in Diet 2B as a source of N to make Diets 2B, 2C, and 2D isonitrogenous, because amino acids (including glutamine and asparagine) have been shown to (positively or negatively)

Table 5. Composition of diets in Experiment 2 (as-fed basis).

Ingredient	Diet 2A Control	Diet 2B Low-CP	Diet 2C Diet 2B + 1% Gln	Diet 2D Diet 2B + 1% Asn
	%	%	%	%
Corn	49.17	64.38	64.38	64.38
Soybean meal (48% CP)	39.90	24.00	24.00	24.00
Soybean oil	6.85	4.68	4.68	4.68
Dicalcium phosphate	1.74	1.86	1.86	1.86
Limestone	1.32	1.36	1.36	1.36
Solka-floc™	–	0.29	0.29	0.29
K_2CO_3	–	0.48	0.48	0.48
NaCl (iodized)	0.25	0.18	0.18	0.18
Na_2CO_3	–	0.07	0.07	0.07
Mineral premix[1]	0.30	0.30	0.30	0.30
Vitamin premix[2]	0.30	0.30	0.30	0.30
Triammonium citrate	–	1.00	–	–
L-Asparagine	–	–	–	1.00
L-Glutamine	–	–	1.00	–
L-Arginine	–	0.17	0.17	0.17
L-Isoleucine	–	0.09	0.09	0.09
L-Valine	–	0.07	0.07	0.07
L-Lysine·HCl	–	0.22	0.22	0.22
DL-Methionine	0.17	0.33	0.33	0.33
L-Threonine	–	0.22	0.22	0.22
Total	100.00	100.00	100.00	100.00

Asn, asparagine; CP, crude protein; Gln, glutamine.

[1]Supplied per kg of diet: Manganese, 70 mg; zinc, 90 mg; iron (ferrous sulfate), 60 mg; copper, 12 mg; selenium (sodium selenite), 0.15 mg; sodium chloride, 2.5 g.

[2]Supplied per kg of diet: Vitamin A (retinyl acetate), 8065 IU; cholecalciferol, 1580 IU; vitamin E (DL-α-tocopheryl acetate), 15 IU; vitamin B_{12}, 16 μg; vitamin K (menadione sodium bisulfite), 4 mg; riboflavin, 7.8 mg; pantothenic acid, 12.8 mg; niacin, 75 mg; choline, 509 mg; folic acid, 1.62 mg; biotin, 270 μg.

affect the activity of ODC (Minami et al., 1985; Rinehart et al., 1985). Lee and Blair (1972) showed that levels up to 4.4% dietary TAC could serve as a nonspecific N source in broiler diets with no adverse effects on growth performance.

Based on calculated values, all four experimental diets met or exceeded NRC (1994) requirements for ME_n, essential amino acids, minerals, and vitamins. The dEB (calculated as Na + K – Cl), crude fiber, and ME_n contents were held constant in all diets to minimize effects of the dietary acid–base balance, fiber, and energy, respectively (Table 6). The CP contents of the ingredients and diets were determined using the micro-Kjeldahl method on a Kjeltech 1028 distilling unit (U.S. Tecator Inc., Herndon, VA, 22070).

Statistical analysis

Data were subjected to ANOVA procedures appropriate for a completely randomized design (Morris, 1999) using the 'Fit Model' command in JMP 4.0.3 (SAS Inst. Inc., Cary, NC). Treatment effects were evaluated as least-squares means and separated by Fischer's protected LSD using StatView 5.0.1 (SAS Inst. Inc., Cary, NC). In addition, the contrast 'Diet 2A vs others' was used to evaluate the effects of the dietary CP level. P-values of 0.05 or less were considered significant.

Experiment 3

Corn and SBM were analyzed for total amino acid contents at a commercial laboratory (Experiment Station Chemical Laboratories, Columbia, MO, 65211) before diet formulation, after which the contents of true digestible amino acids were calculated (Table 7) from digestibility coefficients listed by the NRC (1994). The values for true digestible amino acids were subsequently used for formulation of diets. The CP contents were determined using the micro-Kjeldahl method on a Kjeltech 1028 distilling unit (U.S. Tecator Inc., Herndon, VA, 22070).

Table 6. Chemical composition of diets in Experiment 2 (as-fed basis).

Item	Diet				Requirement[1]	
	2A	2B	2C	2D	True[2]	Total[3]
Analyzed values						
Crude protein (total), %	23.40	19.17	19.26	18.90	–	23.00
Calculated values						
Crude protein (total), %	23.22	18.64	18.77	18.90	–	23.00
ME_n, Mcal/kg	3.20	3.20	3.20	3.19	–	3.20
Ether extract, %	9.12	7.37	7.37	7.37	–	–
Linoleic acid, %	4.68	3.86	3.86	3.86	–	1.00
Crude fiber, %	2.64	2.64	2.64	2.64	–	–
Calcium, %	1.00	1.00	1.00	1.00	–	1.00
Phosphorus (available), %	0.45	0.45	0.45	0.45	–	0.45
Potassium, %	0.94	0.94	0.94	0.94		0.30
Sodium, %	0.20	0.20	0.20	0.20		0.20
Chloride, %	0.33	0.33	0.33	0.33		0.20
Dietary electrolyte balance,[4] %	0.82	0.82	0.82	0.82	–	–
Corn:soybean meal ratio	1.23	2.68	2.68	2.68	–	–
CP_{EAA}:CP_{total} ratio,[5] %	53.0	51.1	50.8	50.5	–	–
True digestible amino acids[6]						
Arginine, %	1.42	1.13	1.13	1.13	1.13	1.25
Glycine, %	0.85	0.60	0.60	0.60	–	–
Glycine + serine, %	1.66	1.17	1.17	1.17	1.13	1.25
Histidine, %	0.56	0.40	0.40	0.40	0.32	0.35
Isoleucine, %	0.94	0.72	0.72	0.72	0.72	0.80
Leucine, %	1.70	1.27	1.27	1.27	1.08	1.20
Lysine, %	1.24	0.99	0.99	0.99	0.99	1.10
Methionine, %	0.49	0.56	0.56	0.56	0.45	0.50
Methionine + cysteine, %	0.81	0.81	0.81	0.81	0.81	0.90
Phenylalanine, %	1.06	0.74	0.74	0.74	0.65	0.72
Phenylalanine + tyrosine, %	1.74	1.21	1.21	1.21	1.21	1.34
Serine, %	0.81	0.57	0.57	0.57	–	–
Threonine, %	0.72	0.72	0.72	0.72	0.72	0.80
Tryptophan, %	0.29	0.19	0.19	0.19	0.18	0.20
Valine, %	1.05	0.81	0.81	0.81	0.81	0.90

ME_n, nitrogen-corrected metabolizable energy.

[1] Source: NRC (1994).

[2] Calculated as 90% of the requirement for total amino acids (see text).

[3] Total amino acids (NRC, 1994).

[4] K + Na – Cl.

[5] Percent of crude protein originating from essential amino acids (proline was included with the nonessential amino acids).

[6] Dietary content calculated using digestibility coefficients from NRC (1994) and Heartland Lysine (1998).

Table 7. Analyzed amino acid and crude protein concentrations of the corn and soybean meal used in Experiment 3 (as-fed basis).

Amino acid	Corn		Soybean meal	
	Total[1] %	True digestible[2] %	Total[1] %	True digestible[2] %
Arginine	0.32	0.28	3.46	3.18
Cysteine	0.17	0.14	0.80	0.66
Glycine[3]	0.27	0.24	1.97	1.75
Histidine	0.19	0.18	1.27	1.12
Isoleucine	0.22	0.19	2.15	2.00
Leucine	0.74	0.69	3.63	3.34
Lysine	0.23	0.19	3.01	2.74
Methionine	0.15	0.14	0.71	0.65
Phenylalanine	0.30	0.27	2.35	2.23
Proline[3]	0.56	0.50	2.34	2.08
Serine[3]	0.28	0.25	2.07	1.84
Threonine	0.24	0.20	1.86	1.64
Tryptophan	0.05	0.04	0.70	0.61
Tyrosine	0.19	0.17	1.66	1.48
Valine	0.32	0.28	2.33	2.12
Crude protein[4]	6.80	–	49.07	–

[1]Total amino acid content.
[2]Calculated from true digestibility coefficients (NRC, 1994) and used in formulation of Diets 3A through 3E.
[3]The true digestibility coefficient was calculated as the mean of all other amino acids' true digestibility coefficients.
[4]Kjeldahl N × 6.25.

A common diet (similar to Diet 3A), formulated with corn and SBM to meet or exceed NRC (1994) requirements, was fed to all the chicks from d 1 to 7 posthatching. From d 7 to 21 posthatching, the chicks were fed one of the five treatment diets, which were formulated to meet or exceed the NRC (1994) requirements for energy, minerals, and vitamins (Table 8). A positive control diet (Diet 3A) was formulated using corn, SBM, and crystalline DL-methionine to meet the NRC (1994) recommendations of 23% CP and 3200 kcal ME_n/kg diet. A negative control diet (Diet 3B) was formulated to contain 18.5% CP using corn, SBM, and crystalline amino acids to meet the true digestible amino acid requirement (i.e., 89% of the requirement for total amino acids). To ensure that the dietary amino acid concentrations were not deficient, the requirement for total amino acids was set at 105% of the NRC (1994) concentrations. Diets 3C, 3D, and 3E were formulated to contain, respectively, 15, 30, and 45% more amino acids

Table 8. Composition of diets in Experiment 3 (as-fed basis).

Ingredient	Diet 3A Control	Diet 3B Low-CP	Diet 3C Diet 3B + 15% cAA	Diet 3D Diet 3B + 30% cAA	Diet 3E Diet 3B + 45% cAA
	%	%	%	%	%
Corn	49.00	62.14	62.14	62.14	62.14
Soybean meal (48% CP)	39.82	26.45	26.45	26.45	26.45
Soybean oil	6.93	4.98	4.98	4.98	4.98
Dicalcium phosphate	1.74	1.83	1.83	1.83	1.83
Limestone	1.32	1.36	1.36	1.36	1.36
Solka-floc™	–	0.23	0.23	0.23	0.23
K_2CO_3	–	0.40	0.40	0.40	0.40
NaCl (iodized)	0.40	0.32	0.31	0.29	0.28
Na_2CO_3	–	0.07	0.09	0.10	0.11
Mineral premix[1]	0.30	0.30	0.30	0.30	0.30
Vitamin premix[2]	0.30	0.30	0.30	0.30	0.30
L-Arginine	–	0.153	0.176	0.199	0.222
L-Glutamate	–	0.500	0.320	0.170	–
L-Isoleucine	–	0.101	0.116	0.131	0.146
L-Lysine·HCl	–	0.239	0.275	0.311	0.347
DL-Methionine	0.190	0.324	0.373	0.421	0.470
L-Threonine	–	0.193	0.222	0.251	0.280
L-Valine	–	0.108	0.124	0.140	0.157
Total	100.00	100.00	100.00	100.00	100.00

cAA, crystalline (essential) amino acids; CP, crude protein.

[1] Supplied per kg of diet: Manganese, 70 mg; zink, 90 mg; iron (ferrous sulfate), 60 mg; copper, 12 mg; selenium (sodium selenite), 0.15 mg; sodium chloride, 2.5 g.

[2] Supplied per kg of diet: Vitamin A (retinyl acetate), 6065 IU; cholecalciferol, 1580 IU; vitamin E (DL-α-tocopheryl acetate), 15 IU; vitamin B_{12}, 16 µg; vitamin K (menadione sodium bisulfite), 4 mg; riboflavin, 7.8 mg; pantothenic acid, 12.8 mg; niacin, 75 mg; choline, 509 mg; folic acid, 1.62 mg; biotin, 270 µg.

from the crystalline sources added in Diet 3B, replacing crystalline L-glutamate (Tables 8 and 9). The CP contents of the diets were determined using the micro-Kjeldahl method on a Kjeltech 1028 distilling unit (U.S. Tecator Inc., Herndon, VA, 22070).

Based on calculated values, all five treatment diets met or exceeded NRC (1994) requirements for ME_n, essential amino acids, minerals, and vitamins. The dEB, crude fiber, and ME_n contents were held constant in all diets to minimize potential effects of the dietary acid–base balance, fiber, and energy, respectively (Table 9).

Table 9. Calculated chemical composition of diets in Experiment 3 (as-fed basis).

Item	Diet					Requirement[1]	
	3A	3B	3C	3D	3E	True[2]	Total[3]
Analyzed values							
Crude protein (total), %	23.99	18.53	18.48	18.66	18.63	–	23.00
Calculated values							
Crude protein (total), %	22.98	18.50	18.55	18.60	18.65	–	23.00
ME_n, Mcal/kg	3.20	3.20	3.20	3.20	3.20	–	3.20
Ether extract, %	9.19	7.61	7.61	7.61	7.61	–	–
Linoleic acid, %	4.72	3.97	3.97	3.97	3.97	–	1.00
Crude fiber, %	2.63	2.63	2.63	2.63	2.63	–	–
Calcium, %	1.00	1.00	1.00	1.00	1.00	–	1.00
Phosphorus (available), %	0.45	0.45	0.45	0.45	0.45	–	0.45
Potassium, %	0.94	0.94	0.94	0.94	0.94	–	0.30
Sodium, %	0.26	0.26	0.26	0.26	0.26	–	0.20
Chloride, %	0.42	0.42	0.42	0.42	0.42	–	0.20
Dietary electrolyte balance,[4] %	0.78	0.78	0.78	0.78	0.78	–	–
Corn:soybean meal ratio	1.23	2.35	2.35	2.35	2.35	–	–
CP_{EAA}:CP_{Total} ratio,[5] %	54.6	55.9	56.7	57.5	60.4	–	–
True digestible amino acids[6]							
Arginine, %	1.41	1.17	1.19	1.21	1.24	1.13	1.25
Glycine, %	0.90	0.67	0.67	0.67	0.67	–	–
Glycine + serine, %	1.84	1.37	1.37	1.37	1.37	1.13	1.25
Histidine, %	0.53	0.41	0.41	0.41	0.41	0.32	0.35
Isoleucine, %	0.89	0.75	0.76	0.78	0.79	0.72	0.80
Leucine, %	1.67	1.31	1.31	1.31	1.31	1.08	1.20
Lysine, %	1.18	1.03	1.06	1.09	1.11	0.99	1.10
Methionine, %	0.51	0.58	0.63	0.67	0.72	0.45	0.50
Methionine + cysteine, %	0.84	0.84	0.89	0.94	0.99	0.81	0.90
Phenylalanine, %	1.02	0.76	0.76	0.76	0.76	0.65	0.72
Phenylalanine + tyrosine, %	1.78	1.32	1.32	1.32	1.32	1.21	1.34
Threonine, %	0.75	0.75	0.78	0.81	0.83	0.72	0.80
Tryptophan, %	0.26	0.19	0.19	0.19	0.19	0.18	0.20
Valine, %	0.98	0.84	0.86	0.87	0.89	0.81	0.90
Amino acids originating from crystalline sources							
Arginine, %	–	0.1499	0.1724	0.1949	0.2174	–	–
Isoleucine, %	–	0.0990	0.1138	0.1287	0.1435	–	–
Lysine, %	–	0.1883	0.2166	0.2448	0.2731	–	–
Methionine, %	0.1851	0.3208	0.3689	0.4170	0.4651	–	–
Threonine, %	–	0.1901	0.2186	0.2471	0.2757	–	–
Valine, %	–	0.1058	0.1217	0.1376	0.1535	–	–

ME_n, nitrogen-corrected metabolizable energy.

[1] Source: NRC (1994).

[2] Calculated as 89% of the requirement for total amino acids (see text).

[3] Total amino acids (NRC, 1994).

[4] K + Na – Cl.

[5] Percent of crude protein originating from essential amino acids (proline was included with the nonessential amino acids).

[6] Dietary content calculated using digestibility coefficients from NRC (1994) and Heartland Lysine (1998).

Statistical analysis

Data were subjected to ANOVA procedures appropriate for a completely randomized design (Morris, 1999) using the 'Fit Model' command in JMP 4.0.3 (SAS Inst. Inc., Cary, NC). Linear and quadratic contrasts were used to evaluate the effects of increasing amounts of crystalline amino acids (i.e., Diets 3B to 3E). In addition, contrasts were used to evaluate the effects of dietary CP level (i.e., Diet 3A vs others). P-values of 0.05 or less were considered significant.

Experiment 4

Corn and SBM were analyzed for total amino acid contents at a commercial laboratory (Experiment Station Chemical Laboratories, Columbia, MO, 65211) before diet formulation, after which the contents of true digestible amino acids were calculated (Table 10) from digestibility coefficients listed by the NRC (1994) and subsequently used for formulation of the diets. The dietary CP contents were determined using the micro-Kjeldahl method on a Kjeltech 1028 distilling unit (U.S. Tecator Inc., Herndon, VA, 22070).

A common corn–SBM diet (similar to Diet 4A), formulated to meet or exceed NRC (1994) requirements, was fed to all the chicks from d 1 to 7 posthatching. From d 7 to 21 posthatching, chicks were fed one of the six treatment diets, which were formulated to meet or exceed the NRC (1994) requirements for energy, minerals, and vitamins (Table 11). A positive control diet (Diet 4A) was formulated using corn, SBM, and crystalline DL-methionine to meet the NRC (1994) recommendations of 23% CP and 3200 kcal ME_n/kg diet. A negative control diet (Diet 4B) was formulated to contain 17.6% CP using corn, SBM, and crystalline amino acids to meet the true digestible amino acid requirement (i.e., 89% of the requirement of total amino acids). To ensure that the dietary amino acid concentrations were not deficient, the requirement for total amino acids was set at 105% of the NRC (1994) concentrations. Diets 4C, 4D, and 4E were

Table 10. Analyzed amino acid and crude protein concentrations of the corn and soybean meal used in Experiment 4 (as-fed basis).

Item	Corn		Soybean meal	
	Total[1] %	True digestible[2] %	Total[1] %	True digestible[2] %
Arginine	0.34	0.30	3.50	3.22
Cysteine	0.18	0.15	0.78	0.64
Glycine[3]	0.27	0.24	1.93	1.72
Histidine	0.20	0.19	1.27	1.12
Isoleucine	0.23	0.20	2.13	1.98
Leucine	0.86	0.80	3.66	3.37
Lysine	0.23	0.19	2.98	2.71
Methionine	0.16	0.15	0.69	0.63
Phenylalanine	0.35	0.32	2.42	2.30
Proline[3]	0.64	0.57	2.39	2.13
Serine[3]	0.31	0.27	1.98	1.76
Threonine	0.25	0.21	1.82	1.60
Tryptophan	0.06	0.05	0.72	0.63
Tyrosine	0.23	0.20	1.69	1.50
Valine	0.33	0.29	2.23	2.03
Crude protein[4]	6.80	–	49.07	–

[1]Total amino acid content.
[2]Calculated from true digestibility coefficients (NRC, 1994) and used in formulation of Diets 3A through 3E.
[3]The true digestibility coefficient was calculated as the mean of all other amino acids' true digestibility coefficients.
[4]Values from Experiment 3 (Table 7).

formulated to contain, respectively, 1, 2, and 3% more crystalline nonessential amino acids (from an equal mix of L-aspartate and L-glutamate) than Diet 4B, replacing cornstarch (Table 11). A sixth diet (Diet 4F) was included in the experiment to investigate whether a combination of insufficient amounts of nonessential amino acids and a less than 100% utilization of crystalline amino acids was responsible for the lower performance of chicks fed low-CP diets. Hence, Diet 4F contained 2% crystalline nonessential amino acids and the concentrations of crystalline essential amino acids were increased by 45% compared with Diet 4B. The CP contents of the diets were determined using the micro-Kjeldahl method on a Kjeltech 1028 distilling unit (U.S. Tecator Inc., Herndon, VA, 22070).

Based on calculated values, all six experimental diets met or exceeded NRC (1994) requirements for ME_n, essential amino acids, minerals, and vitamins. The dEB, crude fiber,

and ME_n contents were held constant in all diets to minimize potential effects of the dietary acid–base balance, fiber, and energy, respectively (Table 12).

Statistical analysis

Data were subjected to ANOVA procedures appropriate for a completely randomized design (Morris, 1999) using the 'Fit Model' command in JMP 4.0.3 (SAS Inst. Inc., Cary, NC). The nonessential amino acids that were added in Diets 4C, 4D, and 4E replaced cornstarch—not a N source—in Diet 4B (Table 11). The dietary CP content therefore increased with higher concentrations of added nonessential amino acids (Table 12). In spite of the increasing CP

Table 11. Composition of diets in Experiment 4 (as-fed basis).

Ingredient	Diet 4A Control	Diet 4B Low-CP	Diet 4C Diet 4B + 1% NEAA	Diet 4D Diet 4B + 2% NEAA	Diet 4E Diet 4B + 3% NEAA	Diet 4F Diet 4D + 45% cAA
	%	%	%	%	%	%
Corn	48.20	60.14	60.14	60.14	60.14	60.14
Cornstarch	–	3.81	2.54	1.27	–	0.68
Soybean meal (48% CP)	40.50	25.15	25.15	25.15	25.15	25.15
Soybean oil	7.05	4.55	4.82	5.09	5.36	5.09
Dicalcium phosphate	1.74	1.86	1.86	1.86	1.86	1.86
Limestone	1.32	1.35	1.35	1.35	1.35	1.35
Solka-floc™	–	0.34	0.34	0.34	0.34	0.34
K_2CO_3	–	0.47	0.47	0.47	0.47	0.47
NaCl (iodized)	0.40	0.30	0.30	0.30	0.30	0.30
Na_2CO_3	–	0.09	0.09	0.09	0.09	0.09
Mineral premix[1]	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin premix[2]	0.30	0.30	0.30	0.30	0.30	0.30
L-Arginine	–	0.18	0.18	0.18	0.18	0.26
L-Aspartate	–	–	0.50	1.00	1.50	1.00
L-Glutamate	–	–	0.50	1.00	1.50	1.00
L-Isoleucine	–	0.13	0.13	0.13	0.13	0.19
L-Lysine·HCl	–	0.30	0.30	0.30	0.30	0.43
DL-Methionine	0.19	0.35	0.35	0.35	0.35	0.50
L-Threonine	–	0.22	0.22	0.22	0.22	0.32
L-Valine	–	0.16	0.16	0.16	0.16	0.23
Total	100.00	100.00	100.00	100.00	100.00	100.00

cAA, crystalline (essential) amino acids; CP, crude protein; NEAA, nonessential amino acids.

[1]Supplied per kg of diet: Manganese, 70 mg; zink, 90 mg; iron (ferrous sulfate), 60 mg; copper, 12 mg; selenium (sodium selenite), 0.15 mg; sodium chloride, 2.5 g.

[2]Supplied per kg of diet: Vitamin A (retinyl acetate), 8065 IU; cholecalciferol, 1580 IU; vitamin E (DL-α-tocopheryl acetate), 15 IU; vitamin B_{12}, 16 µg; vitamin K (menadione sodium bisulfite), 4 mg; riboflavin, 7.8 mg; pantothenic acid, 12.8 mg; niacin, 75 mg; choline, 509 mg; folic acid, 1.62 mg; biotin, 270 µg.

Table 12. Chemical composition of diets in Experiment 4 (as-fed basis).

Item	Diet						Requirement[1]	
	4A	4B	4C	4D	4E	4F	True[2]	Total[3]
Analyzed values								
Crude protein (total), %	23.37	18.26	18.71	19.43	20.02	20.27	–	23.00
Calculated values								
Crude protein (total), %	23.26	17.64	18.25	18.87	19.48	19.40	–	23.00
Crude protein from EAA, %	12.79	10.28	10.28	10.28	10.28	10.77	–	–
Crude protein from NEAA, %	10.47	7.36	7.98	8.59	9.20	8.63	–	–
ME_n, Mcal/kg	3.20	3.20	3.20	3.20	3.20	3.20	–	3.20
Ether extract, %	9.29	7.10	7.36	7.63	7.90	7.63	–	–
Linoleic acid, %	4.76	3.71	3.84	3.98	4.11	3.98	–	1.00
Crude fiber, %	2.64	2.64	2.64	2.64	2.64	2.64	–	–
Calcium, %	1.00	1.00	1.00	1.00	1.00	1.00	–	1.00
Phosphorus, %	0.45	0.45	0.45	0.45	0.45	0.45	–	0.45
Potassium, %	0.95	0.95	0.95	0.95	0.95	0.95	–	0.30
Sodium, %	0.26	0.26	0.26	0.26	0.26	0.26	–	0.20
Chloride, %	0.42	0.42	0.42	0.42	0.42	0.44	–	0.20
Dietary electrolyte balance,[4] %	0.80	0.80	0.80	0.80	0.80	0.77	–	–
Corn:soybean meal ratio	1.19	2.39	2.39	2.39	2.39	2.39	–	–
CP_{EAA}:CP_{Total} ratio,[5] %	55.0	58.3	56.3	54.5	52.8	55.5	–	–
True digestible amino acids[6]								
Arginine, %	1.45	1.17	1.17	1.17	1.17	1.25	1.13	1.25
Glycine, %	0.90	0.63	0.63	0.63	0.63	0.63	–	–
Glycine + serine, %	1.83	1.29	1.29	1.29	1.29	1.29	1.13	1.25
Histidine, %	0.54	0.39	0.39	0.39	0.39	0.39	0.32	0.35
Isoleucine, %	0.90	0.75	0.75	0.75	0.75	0.81	0.72	0.80
Leucine, %	1.75	1.33	1.33	1.33	1.33	1.33	1.08	1.20
Lysine, %	1.19	1.03	1.03	1.03	1.03	1.13	0.99	1.10
Methionine, %	0.51	0.59	0.59	0.59	0.59	0.74	0.45	0.50
Methionine + cysteine, %	0.85	0.84	0.84	0.84	0.84	1.00	0.81	0.90
Phenylalanine, %	1.08	0.77	0.77	0.77	0.77	0.77	0.65	0.72
Phenylalanine + tyrosine, %	1.88	1.33	1.33	1.33	1.33	1.33	1.21	1.34
Threonine, %	0.75	0.75	0.75	0.75	0.75	0.85	0.72	0.80
Tryptophan, %	0.28	0.19	0.19	0.19	0.19	0.19	0.18	0.20
Valine, %	0.96	0.84	0.84	0.84	0.84	0.91	0.81	0.90
Amino acids originating from crystalline sources								
Arginine, %	–	0.1764	0.1764	0.1764	0.1764	0.2558	–	–
Aspartate, %	–	–	0.4900	0.9800	1.4700	0.9800	–	–
Glutamate, %	–	–	0.4900	0.9800	1.4700	0.9800	–	–
Isoleucine, %	–	0.1284	0.1284	0.1284	0.1284	0.1862	–	–
Lysine, %	–	0.2340	0.2340	0.2340	0.2340	0.3394	–	–
Methionine, %	0.1851	0.3416	0.3416	0.3416	0.3416	0.4952	–	–
Threonine, %	–	0.2187	0.2187	0.2187	0.2187	0.3171	–	–
Valine, %	–	0.1568	0.1568	0.1568	0.1568	0.2274	–	–

EAA, essential amino acids; ME_n, nitrogen-corrected metabolizable energy; NEAA, nonessential amino acids.

[1]Source: NRC (1994).

[2]Calculated as 89% of the requirement for total amino acids (see text).

[3]Total amino acid (NRC, 1994).

[4]K + Na – Cl.

[5]Percent of crude protein originating from essential amino acids (proline was included with the nonessential amino acids).

[6]Dietary content calculated using digestibility coefficients from NRC (1994) and Heartland Lysine (1998).

concentration, the dietary CP concentrations were, nevertheless, lower than the control diet, so the contrast 'Diet 4A vs Diets 4B, 4C, 4D, and 4E' was deemed appropriate to evaluate potential differences between the control and low-CP diets. Because the dietary additions of nonessential amino acids in Diets 4B, 4C, 4D, and 4E increased linearly, linear and quadratic contrasts were also used to evaluate potential effects. Furthermore, Diet 4A was compared with Diet 4E (containing the highest amount of added nonessential amino acids) using contrasts. Because of the combination of increased concentrations of both crystalline essential and nonessential amino acids in Diet 4F, this diet was examined separately from Diets 4B, 4C, 4D, and 4E. Effects of Diet 4F were compared with the positive control diet, Diet 4A, using contrasts. P-values of 0.05 or less were considered significant in all comparisons.

RESULTS AND DISCUSSION

Experiment 1

Experiment 1 was designed to investigate the effects of the molecular form of protein (i.e., intact protein vs free amino acids) on growth performance and N retention of broiler chicks. Diets 1A and 1B were formulated to contain equal amounts of protein (albeit in different molecular form). However, Diet 1A contained more total CP (24.6%) than did Diet 1B (22.3%), a result of the more digestible protein in Diet 1B (about half the protein in Diet 1B originated from crystalline amino acids, which were assumed 100% digestible). Applying an average coefficient for true digestibility of 0.89 for amino acids in corn and SBM (NRC, 1994), the true digestible CP concentration in Diet 1A was 21.9%, which matched the 22.3% CP in Diet 1B fairly well.

The amino acid contents of the diets in Experiment 1 were analyzed and are presented in Table 13. At first glance, the diets did not seem to meet the NRC (1994) requirements for total amino acids; however, the diets were formulated on a true digestible amino acid basis (not total amino acids). Furthermore, a relatively large proportion of the amino acids in Diets 1B, 1C, and 1D was from crystalline sources, which reportedly are of higher true digestibility than the amino acids in corn and SBM (Izquierdo et al., 1988; Chung and Baker, 1992; NRC, 1994). Thus, the concentrations of total amino acids in the diets were expected to be lower than that recommended by the NRC (1994)—especially if the amino acids were added in crystalline form because of differences in digestibility between crystalline amino acids (100% digestible) and amino acids in corn and SBM (89% digestible on average). Calculations of dietary contents and requirements for true digestible amino acids, are shown in the Appendix and reported in Table 13. From observation of the results in Table 13, it was evident that the concentrations of true digestible methionine + cysteine, glycine + serine, phenylalanine + tyrosine, isoleucine, and

Table 13. Amino acid composition of the diets in Experiment 1 (as-fed basis).

Amino acid	Total amino acids[1]					True digestible amino acids[1]				
	Analyzed[2]				Required[3]	Calculated from analyzed contents[4]				Required[5]
	Diet 1A	Diet 1B	Diet 1C	Diet 1D		Diet 1A	Diet 1B	Diet 1C	Diet 1D	
	%	%	%	%	%	%	%	%	%	%
Alanine	0.99	1.00	0.51	0.69	–	0.87	0.94	0.44	0.61	–
Arginine	1.51	1.39	1.35	1.27	1.25	1.37	1.33	1.29	1.16	1.13
Aspartate + asparagine	2.18	2.20	1.20	1.60	–	1.91	2.06	1.05	1.40	–
Cysteine	0.46	0.28	0.23	0.27	–	0.37	0.25	0.19	0.22	–
Glutamate + glutamine	3.89	3.82	1.99	2.78	–	3.40	3.58	1.74	2.43	–
Glycine + serine	1.99	2.00	**1.15**	1.42	1.25	1.74	1.87	**1.02**	1.24	1.09
Histidine	0.60	0.59	0.58	0.47	0.35	0.53	0.55	0.54	0.41	0.31
Isoleucine	0.85	0.86	0.86	**0.73**	0.80	0.76	0.81	0.81	**0.66**	0.71
Leucine	1.72	1.80	1.75	1.28	1.20	1.61	1.74	1.69	1.19	1.12
Lysine	1.19	1.18	1.17	**1.07**	1.10	0.99	1.07	1.07	0.93	0.92
Methionine	**0.42**	**0.44**	0.55	0.54	0.50	**0.40**	**0.42**	0.54	0.52	0.46
Methionine + cysteine	**0.88**	**0.72**	**0.78**	**0.81**	0.90	0.78	**0.74**	**0.74**	**0.76**	0.78
Phenylalanine	1.02	1.07	1.37	0.74	0.72	0.93	1.02	1.33	0.67	0.66
Phenylalanine + tyrosine	1.79	1.84	1.77	**1.28**	1.34	1.62	1.74	1.68	**1.16**	1.21
Proline[1]	–	–	–	–	0.60	–	–	–	–	0.53
Threonine	0.80	**0.77**	**0.75**	**0.76**	0.80	0.68	0.70	0.69	0.67	0.67
Tyrosine	0.77	0.77	0.40	0.55	–	0.69	0.73	0.35	0.49	–
Tryptophan[1]	–	–	–	–	0.20	–	–	–	–	0.16
Valine	0.91	0.94	0.92	**0.79**	0.90	0.80	0.88	0.86	**0.71**	0.79

–, Not analyzed or no requirement listed by the NRC (1994). **Values in bold** were below the NRC (1994) requirements for total amino acids and/or the estimated requirements for true digestible amino acids.

[1] Proline and tryptophan were not analyzed.

[2] Analyzed total dietary amino acid content.

[3] Requirements for total amino acids (NRC, 1994).

[4] Calculated from the analyzed total amino acid content (see the Appendix).

[5] Calculated from the NRC (1994) listed total amino acid requirement (see the Appendix).

valine were slightly below the estimated requirements in some of the diets. That said, the calculated concentrations of nonessential true digestible amino acids in Diets 1A and 1B, and the calculated concentrations of true digestible essential amino acids in Diets 1A, 1B, and 1C, matched fairly well (Table 13). Diet 1D was included in the experiment as a negative control diet and its concentrations of (true digestible) amino acids were not necessarily meant to equal the concentrations found in Diets 1A, 1B, and 1C.

The observed room temperature was higher than the planned 21°C, with an average temperature of 26.6 ± 0.1°C from d 7 to 21 posthatching (i.e., during the time when the treatment diets were fed). Between d 7 and 21 posthatching, three chicks (one each from Diets 1A, 1B, and 1C) died of reasons considered unrelated to the dietary treatments.

In spite of similar amino acid composition of Diets 1A and 1B, growth performance differed between chicks fed the two diets (Table 14). Chicks fed Diet 1A, the positive control diet, grew faster ($P < 0.05$) and utilized the feed more efficiently ($P < 0.05$) than did chicks fed any of the other diets. Chicks fed Diet 1B consumed less feed ($P < 0.05$) than did chicks fed Diet 1A, which may have led to the lower ($P < 0.05$) ADG. However, G:F was lower ($P < 0.05$) in chicks fed Diet 1B compared with chicks fed the intact-protein diet, Diet 1A, suggesting that factors other than a lower feed intake were responsible for the lower ADG. Differences in growth performance between Diets 1A and 1B could potentially be attributed to Diet 1B's lower concentration of the sulfur amino acids, methionine + cysteine, which are considered the first-limiting amino acids for poultry (Table 13). However, the relatively small difference in dietary methionine + cysteine between the two diets (5%) probably cannot account for the total difference in growth performance, which amounted to 14, 8, and 6% for ADG, ADFI, and G:F, respectively (calculated from data in Table 14).

Table 14. Growth performance[1] (Experiment 1).

Item[2]	Diet 1A Intact protein (positive control)	Diet 1B Crystalline essential and nonessential amino acids	Diet 1C Crystalline essential amino acids	Diet 1D Low-CP (negative control)	SE	P[3]
Average daily gain, g/d						
d 7–14	31.69[a]	28.16[b]	22.35[c]	27.66[b]	0.49	0.001
d 14–21	53.14[a]	44.99[b]	36.80[c]	44.98[b]	0.61	0.001
d 7–21	42.41[a]	36.58[b]	29.57[c]	36.32[b]	0.47	0.001
Average daily feed intake, g/d						
d 7–14	41.61[a]	39.71[a]	36.85[b]	41.26[a]	0.67	0.001
d 14–21	75.26[a]	67.55[b]	65.15[b]	72.95[a]	1.28	0.001
d 7–21	58.43[a]	53.63[b]	51.00[c]	57.11[a]	0.85	0.001
Feed utilization (gain-to-feed ratio), g/kg						
d 7–14	762[a]	710[b]	607[c]	670[d]	9	0.001
d 14–21	706[a]	667[b]	566[c]	617[d]	12	0.001
d 7–21	726[a]	682[b]	581[c]	636[d]	8	0.001

CP, crude protein; d, day; SE, standard error.

[1]Means of six pens.

[2]Days (d) refer to days posthatching (a common diet was fed from d 1 to 7 posthatching).

[3]P-value of the main effect.

[a,b,c,d]Means within a row lacking a common superscript differ ($P < 0.05$); estimated by Fischer's protected least significant difference.

Because of the differences in ADG, differences ($P < 0.05$) in BW were observed at the end of the trial (Table 15). Thus, the heaviest chicks on d 21 posthatching were those fed Diet 1A, the positive control diet based on intact protein. No difference ($P > 0.10$) was observed in percent whole-body CP between chicks fed Diets 1A and 1B on d 21 posthatching. Because of their greater BW, chicks fed Diet 1A had an increased ($P < 0.05$) whole-body CP content of almost 10% (Table 15), which can be interpreted as 10% more muscle (meat) than that of chicks fed Diet 1B. The higher CP content in chicks fed Diet 1A led to a greater ($P < 0.05$) N retention compared with chicks fed Diet 1B (the whole-body N content on d 7 posthatching did not influence the N retention significantly when included in the statistical analysis as a covariant). The predicted whole-body fat content and fat retention were similar ($P > 0.10$) in chicks fed Diet 1A and Diet 1B (Table 15). Although numerically larger ($P > 0.10$), whole-body fat content at d 7 posthatching did not affect fat retention ($P > 0.10$) when used as a covariant.

Table 15. Body weights and whole-body composition[1] (Experiment 1).

Item[2]	Diet 1A Intact protein (control)	Diet 1B Crystalline essential and nonessential amino acids	Diet 1C Crystalline essential amino acids	Diet 1D Low-CP (negative control)	SE	P[3]
Initial weight (d 7), g	122.03	120.40	120.22	120.04	1.44	0.753
End weight (d 21), g	715.83[a]	632.48[b]	534.23[c]	628.50[b]	7.22	0.001
Whole-body content (d 21)						
Dry matter, %	28.50[a]	28.68[a]	30.06[ab]	30.81[b]	0.54	0.018
Fat,[4] %	6.85[a]	7.09[a]	8.93[ab]	9.91[b]	0.72	0.018
Fat,[4] g	49.05[a]	44.73[a]	47.46[a]	62.47[b]	4.23	0.035
Crude protein, %	15.53	15.93	15.38	15.06	0.21	0.063
Crude protein, g	111.19[a]	100.73[b]	82.15[c]	94.73[d]	1.79	0.001
Fat retention,[4]						
Fat (d 7), g	3.84	3.79	3.78	3.78	0.05	0.761
Fat retention (d 7–21), g	45.21[a]	40.94[a]	43.68[a]	58.69[b]	4.22	0.034
Nitrogen retention						
Whole-body N (d 7), g	2.85	2.81	2.81	2.81	0.03	0.754
Whole-body N (d 21), g	17.79[a]	16.12[b]	13.14[c]	15.16[d]	0.29	0.001
N retention (d 7–21), g	14.94[a]	13.30[b]	10.33[c]	12.35[d]	0.27	0.001
Nitrogen utilization (d 7–21)						
N intake,[5] g	32.19[a]	26.81[b]	21.43[c]	24.11[d]	0.37	0.001
Efficiency,[6] %	46.40[a]	49.62[bc]	48.28[ac]	51.20[b]	0.86	0.006
PER,[7] g/g	2.95[a]	3.06[ab]	3.10[b]	3.38[c]	0.04	0.001
N excretion,[8] g	17.25[a]	13.51[b]	11.10[c]	11.76[c]	0.31	0.001
N excretion,[9] %	53.60[a]	50.38[bc]	51.72[ac]	48.80[b]	0.86	0.006

CP, crude protein; d, day; N, nitrogen; SE, standard error.

[1]Means of six pens.

[2]Days (d) refer to days posthatching (a common diet was fed from d 1 to 7 posthatching).

[3]P-value of main effect.

[4]Whole-body fat content was predicted from the whole-body dry matter content (Velu et al., 1972).

[5]Analyzed dietary N used.

[6]Efficiency of N use (N retention divided by N intake).

[7]Protein efficiency ratio (gain divided by CP intake).

[8]Apparent N excretion (N intake – N retention).

[9]Apparent N excretion as a percentage of N intake.

[abcd]Means within a row lacking a common superscript differ ($P < 0.05$); estimated by Fischer's protected least significant difference.

Chicks fed the intact-protein diet (Diet 1A) had a higher ($P < 0.05$) whole-body content of CP and a higher ($P < 0.05$) N retention than chicks fed Diet 1B, based on free amino acids. The intact protein in Diet 1A supported a higher ($P < 0.05$) growth rate than the crystalline amino acids in Diet 1B (Table 15). However, percent whole-body fat, percent whole-body DM (from which the fat content was predicted), and percent whole-body CP did not differ ($P > 0.10$)

between chicks fed Diets 1A and 1B. Chicks fed Diet 1B utilized N with a higher efficiency ($P < 0.05$) and excreted less N ($P < 0.05$) than chicks fed Diet 1A (Table 15). This response indicated that the crystalline amino acids were utilized more efficiently than amino acids from intact protein. However, the difference is more likely explained by the higher digestibility of the N in Diet 1B due to its content of highly digestible crystalline amino acids. This explanation is validated by the similar ($P = 0.06$) PER resulting from the two diets.

The lower ($P < 0.05$) ADG of chicks fed Diet 1B compared with Diet 1A (Table 14) could reasonably be explained by the lower ($P < 0.05$) ADFI—the chicks consumed less and therefore grew slower. Furthermore, the similarly less efficient ($P < 0.05$) feed utilization (Table 14) may potentially be explained by the lower ADG of chicks fed Diet 1B: A lower ADG may lead to a less efficient feed utilization because the proportion of dietary ME intake that is used for maintenance would be relatively large compared with the proportion used for growth (Lawrence and Fowler, 1997). However, when the dietary ME intakes were calculated for chicks fed Diets 1A and 1B, the proportion of ME intake used to satisfy the maintenance ME requirement to either ME intake available for growth (i.e., total ME intake less the estimated ME requirement) or to total ME intake did not differ between chicks fed Diets 1A and 1B (data not shown; $P > 0.10$). Hence, the relatively less efficient feed utilization of chicks fed Diet 1B could not be attributed to their lower ADG, but rather the molecular form of the dietary protein (i.e., intact protein vs free amino acids). The lower total whole-body CP content in chicks fed Diet 1B suggests that less of the dietary amino acids in Diet 1B were available for protein synthesis (i.e., a slower lean gain). Moreover, the less efficient feed utilization by chicks fed free amino acids indicates that the absorption of free amino acids may come at a higher energetic cost than absorption of peptide-bound amino acids as hypothesized. That said, other factors could influence the feed utilization. Feed wastage could account for the observations, but, although not measured, excessive feed waste was not noticed in any of the pens during Experiment 1. The high dietary content of

cornstarch in Diet 1B compared with that in Diet 1A (Table 2) yielded a more 'powdery' diet, which may have influenced feed intake (Russell and Gahr, 2000). However, a similarly high content of cornstarch in Diet 1D did not significantly lower the feed intake compared with that of Diet 1A (Table 14), suggesting that the physical form of the diet did not influence the chicks' performance.

Chicks fed Diet 1C, which lacked dietary crystalline *nonessential* amino acids, had the poorest ($P < 0.05$) growth performance (Table 14). This response can be attributed in part to slight deficiencies of dietary amino acids (glycine + serine and methionine + cysteine; Table 13) and/or the molecular form of the dietary protein as with Diet 1B. However, a potentially more important contributor to the slower growth was the CP_{EAA}:CP_{Total} ratio of 0.69 (Table 3), well above the optimal 0.55 suggested by Bedford and Summers (1985). Consequently, Diet 1C may have contained too little N from nonessential amino acids, meaning that essential amino acids probably had to be transaminated to supply the N in endogenously synthesized nonessential amino acids. This factor may have further increased the (slight) deficiency of essential amino acids, resulting in the poor growth performance. Omitting dietary crystalline nonessential amino acids (Diet 1C) lowered the final BW, as well as the total amount of whole-body protein ($P < 0.05$) compared with chicks fed Diet 1B. N intake was also significantly lower in chicks fed Diet 1C compared with chicks fed Diet 1B, which was induced by a lower dietary CP content (Table 3), as well as a lower overall ADFI (Table 14). The lower whole-body CP content led to the lowest ($P < 0.05$) N retention of all the diets, but, with the reduced N intake, the efficiency of N use was not affected ($P > 0.10$) compared with chicks fed Diets 1A and 1B.

Chicks fed the low-CP, negative-control diet (Diet 1D) grew slower ($P < 0.05$) than chicks fed Diet 1A (Table 14). Feed intake between those two groups was, however, similar ($P > 0.10$), leading to a significantly lower efficiency of utilization of Diet 1D. Diet 1D was formulated to meet or exceed the NRC (1994) requirements for essential amino acids on a total amino acid

basis and to have a CP_{EAA}:CP_{Total} ratio close to the optimal 0.55 (Table 3). Analyzed values, however, showed a deficiency relative to their requirements of several amino acids (Table 13), which may explain, in part, the poorer growth performance. Chicks fed Diet 1D had a percentage of whole-body CP comparable ($P > 0.10$) to that of chicks fed Diet 1A, but a lower amount of total whole-body CP ($P < 0.05$) caused by the lower BW (Table 15). The percentage and total amount of whole-body fat was higher ($P < 0.05$) than that resulting from Diet 1A, leading to a poorer carcass quality compared with chicks fed Diet 1A. Furthermore, Diet 1D also resulted in a lower N retention than the high-CP diet, Diet 1A. N utilization of chicks fed the low-CP diet and apparent N excretion was, however, superior ($P < 0.05$) to that of chicks fed the control diet, Diet 1A. The relatively higher efficiency of N use and the lower N excretion indicate that the dietary protein in Diet 1D was closer to ideal protein than the protein in Diet 1A.

Experiment 2

Experiment 2 was designed to investigate whether 1% dietary glutamine or 1% dietary asparagine (both replacing 1% TAC) would improve growth performance and N retention of broiler chicks fed low-CP diets. The average room temperature was 23.7 ± 0.7°C from d 7 to 21 posthatching (i.e., during the time when the treatment diets were fed). During the trial, two chicks fed Diet 2D died of reasons considered unrelated to the dietary treatment. A third chick (Diet 2C) was culled due to leg problems.

The feed intake in one pen (fed Diet 2A) was considerably higher than the mean feed intake for that diet on the weigh day on d 14 posthatching (Table 16). The value was not considered representative of the actual feed intake by the 10 chicks in the pen because the ADG of the chicks in the affected pen was similar to the ADG observed in other pens fed Diet 2A (Table 16). The value was considered an outlier after a Q test was performed (Dean and Dixon, 1951) and was not used in calculations of the mean feed intake for Diet 2A. Pen feed intakes for

Table 16. Pen feed intake and body-weight gain of chicks fed Diet 2A from d 7 to 14 posthatching (Experiment 2).

Item	Pen feed intake[1]	Pen gain[1]
	g	g
Pen[2]		
a	2,935	2,351
b	2,995	2,309
c	3,006	2,286
d	3,058	2,398
e	3,121	2,425
f	4,299	2,306
Calculations		
Mean ± SD, pens a–f	3,236 ± 525	2,346 ± 56
Mean ± SD, pens a–e	3,023 ± 70	2,354 ± 58
Median, pens a–f	3,032	2,330

d, day; SD, standard deviation.
[1]Each pen contained 10 chicks.
[2]Pens are listed in ascending order of magnitude in regard to feed intake.

the subsequent week (i.e., d 14 to 21 posthatching) were similar in all pens fed Diet 2A (data not shown). Although not measured, no excessive feed waste was observed in any of the pens during Experiment 2.

No effects on growth performance ($P > 0.10$) were found by replacing 1% TAC with either 1% glutamine or 1% asparagine (Table 17). Asparagine, however, did tend ($P = 0.08$) to improve feed utilization from d 14 to 21 compared with glutamine, but asparagine did not ($P = 0.86$) improve feed utilization compared with TAC in the same week. Thus, TAC was as efficient in supplying dietary non-specific N as were the two nonessential amino acids, glutamine and asparagine. ADG, ADFI, and G:F of chicks fed the control diet (Diet 2A) were superior ($P < 0.05$) to chicks fed any of the low-CP diets, although no differences ($P > 0.10$) were observed in ADFI between d 14 and 21. The lack of differences among the low-CP diets, combined with the lower performance compared with Diet 1A, led to the conclusion that neither 1% L-glutamine nor 1% L-asparagine improved growth performance of chicks fed low-CP diets.

Table 17. Growth performance[1] (Experiment 2).

Item[2]	Diet 2A Control	Diet 2B Low-CP	Diet 2C Diet 2B + 1% Gln	Diet 2D Diet 2B + 1% Asn	SE	P[3]	Contrast[4]
Average daily gain, g/d							
d 7–14	33.51[a]	32.11[c]	32.24[b]	31.97[b]	0.38	0.033	0.004
d 14–21	60.14[a]	58.01[b]	56.37[b]	56.98[b]	0.69	0.006	0.001
d 7–21	46.82[a]	45.06[b]	44.31[b]	44.48[b]	0.50	0.007	0.001
Average daily feed intake, g/d							
d 7–14	43.19[a]	45.43[b]	45.48[b]	45.14[b]	0.41	0.004	0.001
d 14–21	83.58	86.16	84.92	84.50	0.93	0.290	0.146
d 7–21	63.23	65.80	65.20	64.82	0.64	0.083	0.019
Feed utilization (gain-to-feed ratio), g/kg							
d 7–14	779[a]	707[c]	709[b]	708[b]	6	0.001	0.001
d 14–21	720[a]	673[b]	664[b]	674[b]	4	0.001	0.001
d 7–21	739[a]	685[b]	680[b]	686[b]	4	0.001	0.001

Asn, asparagine; CP, crude protein; d, day; Gln, glutamine; SE, standard error.
[1]Least-squares means (n = 6).
[2]Days (d) refer to days posthatching (a common diet was fed from d 1 to 7 posthatching).
[3]P-value of the main effect.
[4]P-value of the contrast 'Diet 2A vs Diets 2B, 2C, and 2D.'
[a,b,c]Means within a row lacking a common superscript differ ($P < 0.05$), estimated by Fischer's protected least significant difference.

As with the growth performance, no differences ($P > 0.10$) were found in whole-body composition, N retention, or N utilization among chicks fed any of the three low-CP diets (Table 18). Differences in ADG (Table 17) were responsible for significantly higher BW at the end of the trial of chicks fed the high-CP diet, Diet 2A, compared with chicks fed any of the low-CP diets (Table 18). Because there was no difference ($P > 0.10$) in percent whole-body CP, the higher BW of chicks fed Diet 2A resulted in a numerically higher amount of CP in the whole body. This difference, however, was not significant when Fisher's protected LSD was used to separate the means because of the relatively large standard error (SE). Only when the contrast 'Diet 2A vs others' was implemented was the effect significant, probably because the SE of the mean of the three low-CP diets together was lower compared with the SE of each of the low-CP diets by themselves. The main effects of the four treatments were not significant for whole-body N content and N retention. However, both measures were numerically higher in the control

group and, when separated using contrasts, were significantly different (Table 18). As in Experiment 1, the low-CP diets (Diets 2B, 2C, and 2D) resulted in an improved ($P < 0.05$) N utilization and PER and a reduced ($P < 0.05$) apparent N excretion. Thus, the protein in the low-CP diets was closer to ideal protein than the protein in Diet 2A so the chicks fed the low-CP diets excreted less N in the manure.

Table 18. Body weights and whole-body composition[1] (Experiment 2).

Item[2]	Diet 2A Control	Diet 2B Low-CP	Diet 2C Diet 2B + 1% Gln	Diet 2D Diet 2B + 1% Asn	SE	P[3]	Contrast[4]
Initial weight (d 7), g	128.70	128.12	128.18	128.08	0.39	0.655	0.219
End weight (d 21), g	784.23[a]	758.98[b]	748.48[b]	750.74[b]	7.13	0.008	0.001
Whole-body content (d 21)							
Dry matter, %	27.88[a]	30.39[b]	30.17[b]	30.37[b]	0.47	0.004	0.001
Fat,[5] %	5.71[a]	9.34[b]	9.06[b]	9.33[b]	0.61	0.001	0.001
Fat,[5] g	44.85[a]	71.00[b]	67.99[b]	69.93[b]	4.78	0.002	0.001
Crude protein, %	16.22	15.94	16.17	16.13	0.23	0.834	0.600
Crude protein, g	127.22	121.00	121.03	121.01	2.18	0.142	0.023
Nitrogen retention							
Whole-body N (d 7), g	3.13	3.12	3.12	3.12	0.01	0.785	0.322
Whole-body N (d 21), g	20.29	19.36	19.37	19.36	0.37	0.250	0.048
N retention (d 7–21), g	17.17	16.24	16.25	16.25	0.36	0.252	0.049
Nitrogen utilization							
N intake (d 7–21),[6] g	33.14[a]	28.26[b]	28.13[b]	27.44[b]	0.29	0.001	0.001
Efficiency,[7] %	51.79[a]	57.49[b]	57.72[b]	59.22[b]	1.01	0.004	0.001
PER[8] (d 7–21), g/g	3.16[a]	3.57[b]	3.51[b]	3.58[b]	0.24	0.001	0.001
N excretion,[9] g	15.97[a]	12.01[b]	11.88[b]	11.19[b]	0.32	0.001	0.001
N excretion,[10] %	48.21[a]	42.51[b]	42.28[b]	40.78[b]	0.99	0.001	0.001

Asn, asparagine; CP, crude protein; d, day; Gln, glutamine; N, nitrogen; SE, standard error.

[1]Least-squares means (n = 6).

[2]Days (d) refer to days posthatching (a common diet was fed from d 1 to 7 posthatching).

[3]P-value of the main effect.

[4]P-value of the contrast 'Diet 2A vs Diets 2B, 2C, and 2D.'

[5]Whole-body fat content was predicted from the whole-body dry matter content (see text).

[6]Analyzed dietary N used.

[7]Efficiency of N use (N retention divided by N intake).

[8]Protein efficiency ratio (gain divided by CP intake).

[9]Apparent N excretion (N intake – N retention).

[10]Apparent N excretion as a percentage of N intake.

[a,b]Means within a row lacking a common superscript differ ($P < 0.05$), estimated by Fischer's protected least significant difference.

The dietary treatments did not have a large effect on the whole-body CP content of the chicks. However, chicks fed any of the three low-CP diets were fatter ($P < 0.001$) both when measured as percent whole-body fat and as total grams of fat, than chicks fed the control diet (Table 18). The reduced growth performance (Table 17), combined with the inferior carcass composition, made Diets 2B, 2C, and 2D impractical to feed unless a large economical value would be placed on minimization of apparent N excretion. Although the concentrations of glutamine and asparagine in corn and SBM are unknown due to the difficulty in the analysis (Nissen, 1992), the lack of response to either 1% dietary glutamine or asparagine indicates that low-CP diets in general contain sufficient amounts of those two amino acids and that further supplementation is not needed.

Experiment 3

Experiment 3 was designed to investigate whether increased dietary concentrations of crystalline essential amino acids would improve growth performance and N retention of broiler chicks fed low-CP diets. The room temperature was 22.7 ± 0.2°C from d 7 to 21 posthatching (i.e., during the time when the treatment diets were fed). Of the 300 chicks that were allotted to the dietary treatments, six chicks (two chicks fed Diet 3B, one chick fed Diet 3C, two chicks fed Diet 3D, and one chick fed Diet 2E) died of reasons considered unrelated to the dietary treatments between d 7 and 21 posthatching. Additionally, two chicks (one each from Diets 2B and 2C) were culled because of leg problems during the course of the trial.

Even though no effect of diet ($P > 0.10$) was observed in ADG during the first week, ADG of chicks fed the low-CP diets (i.e., Diets 3B through 3E) was less than those fed the control diet ($P < 0.01$) in the second week of the trial, as well as in the overall period (Table 19). There were no significant linear or quadratic responses of increasing the dietary concentrations of essential crystalline amino acids on ADG. Feed intake was higher (P 0.01) in chicks fed the

Table 19. Growth performance[1] (Experiment 3).

Item[2]	Diet 3A Control	Diet 3B Low-CP	Diet 3C Diet 3B + 15% cAA	Diet 3D Diet 3B + 30% cAA	Diet 3E Diet 3B + 45% cAA	SE	P[3]	Contrasts		
								CP[4]	L[5]	Q[6]
Average daily gain, g/d										
d 7–14	35.93	36.02	35.47	34.97	34.99	0.45	0.333	0.272	0.090	0.536
d 14–21	68.50	65.08	64.31	63.58	64.15	0.79	0.001	0.001	0.327	0.401
d 7–21	52.21	50.55	49.89	49.28	49.57	0.51	0.004	0.001	0.135	0.361
Average daily feed intake, g/d										
d 7–14	47.11	50.87	50.39	50.05	48.66	0.49	0.001	0.001	0.004	0.363
d 14–21	90.02	93.36	91.98	91.39	90.87	0.91	0.141	0.076	0.058	0.638
d 7–21	68.57	72.11	71.18	70.72	69.77	0.61	0.004	0.002	0.011	0.987
Feed utilization (gain-to-feed ratio), g/kg										
d 7–14	763	708	704	699	719	6	0.010	0.001	0.338	0.069
d 14–21	761	697	699	696	706	5	0.001	0.001	0.305	0.423
d 7–21	762	701	701	697	711	4	0.001	0.001	0.201	0.121

cAA, crystalline amino acids; CP, crude protein; SE, standard error.

[1]Means of six pens.

[2]Days (d) refer to days posthatching (a common diet was fed from d 1 to 7 posthatching).

[3]P-value of the main effect.

[4]P-value of the contrast 'Diet 3A vs Diets 3B, 3C, 3D, and 3E.'

[5]P-value of the linear contrast of Diets 3B, 3C, 3D, and 3E.

[6]P-value of the quadratic contrast of Diets 3B, 3C, 3D, and 3E.

low-CP diets than chicks fed the control diet, except from d 14 to 21, where only a trend (P = 0.08) towards higher ADFI was observed. However, increasing the amounts of dietary crystalline amino acids lowered the overall feed intake linearly (P < 0.01) to that of chicks fed the control diet (Table 19). Feed utilization, on the other hand, was significantly poorer after feeding any of the low-CP diets compared with the control diet (P < 0.01) and did not improve with the added crystalline amino acids (P > 0.10).

As in the previous experiments, the poorer ADG of chicks fed the low-CP diets resulted in lighter (P < 0.01) BW at the end of the trial (Table 20). Whole-body DM was significantly higher in chicks fed low-CP diets compared with those fed the control diet, and was not affected by increasing the dietary concentrations of crystalline essential amino acids (P > 0.10). The higher whole-body DM content led to a (predicted) higher percent fat in chicks fed the low-CP diets as compared with that of chicks fed the control diet. In contrast to Experiments 1 and 2, the low-CP diets in this trial resulted in a lower (P < 0.01) percentage of CP in the whole body as compared with the control-fed chicks. This difference carried over in the absolute amount of CP in the chicks, where the control-fed chicks had a higher amount of CP (P < 0.01). Moreover, contrary to expectations, increasing the concentrations of dietary crystalline amino acids tended (P = 0.07) to *decrease* the amount of CP in the bodies (Table 20). Differences in whole-body N were also responsible for a significant lower N retention in low-CP–fed chicks compared with chicks fed the control diet. Additions of crystalline essential amino acids tended (P = 0.06) to decrease N retention linearly. As in the other experiments, the lower (P < 0.01) N intake by the chicks fed the low-CP diets led to an increase (P < 0.01) in efficiency of N utilization and PER, as well as a decrease (P < 0.001) in apparent N excretion. No effects, however, were observed on N utilization by adding increments of crystalline amino acids to the diets (P > 0.10).

Increasing the concentrations of dietary crystalline essential amino acids by up to 45% above that in the negative control, low-CP diet improved neither growth performance (Table

Table 20. Body weights and whole-body composition[1] (Experiment 3).

Item[2]	Diet 3A Control	Diet 3B Low-CP	Diet 3C Diet 3B + 15% cAA	Diet 3D Diet 3B + 30% cAA	Diet 3E Diet 3B + 45% cAA	SE	P[3]	Contrasts		
								CP[4]	L[5]	Q[6]
Initial weight (d 7), g	146.68	146.48	146.50	145.78	146.35	0.32	0.349	0.289	0.427	0.447
End weight (d 21), g	877.67	854.17	844.96	835.65	840.33	7.07	0.003	0.001	0.121	0.335
Whole-body content (d 21)										
Dry matter, %	28.64	30.73	30.52	31.30	30.37	0.35	0.001	0.001	0.841	0.313
Fat,[7] %	7.03	9.82	9.53	10.56	9.34	0.46	0.001	0.001	0.840	0.315
Fat,[7] g	61.61	83.82	80.59	88.26	78.44	3.94	0.001	0.001	0.635	0.411
Crude protein, %	16.92	16.01	16.02	15.84	15.74	0.16	0.001	0.001	0.181	0.747
Crude protein, g	148.46	136.76	135.32	132.43	132.23	1.87	0.001	0.001	0.066	0.748
Fat retention[7]										
Fat (d 7), g	5.12	5.12	5.12	5.09	5.11	0.01	0.377	0.312	0.377	0.420
Fat retention (d 7–21), g	56.49	78.71	75.47	83.18	73.33	3.94	0.001	0.001	0.637	0.410

Table 20. (continued)

Item[2]	Diet 3A Control	Diet 3B Low-CP	Diet 3C Diet 3B + 15% cAA	Diet 3D Diet 3B + 30% cAA	Diet 3E Diet 3B + 45% cAA	SE	P[3]	Contrasts CP[4]	Contrasts L[5]	Contrasts Q[6]
Nitrogen retention										
Whole-body N (d 7), g	3.51	3.50	3.50	3.49	3.50	0.01	0.350	0.285	0.430	0.444
Whole-body N (d 21), g	23.75	21.88	21.65	21.19	21.16	0.30	0.001	0.001	0.060	0.743
N retention (d 7–21), g	20.25	18.38	18.15	17.70	17.66	0.30	0.001	0.001	0.063	0.758
Nitrogen utilization										
N intake (d 7–21),[8] g	36.84	29.93	29.46	29.56	29.12	0.27	0.001	0.001	0.061	0.955
Efficiency,[9] %	54.96	61.40	61.59	59.89	60.64	0.85	0.001	0.001	0.310	0.745
PER[10] (d 7–21), g/g	3.18	3.69	3.75	3.68	3.80	0.05	0.001	0.001	0.129	0.740
N excretion,[11] g	16.60	11.55	11.32	11.85	11.46	0.28	0.001	0.001	0.842	0.785
N excretion,[12] %	45.04	38.60	38.41	40.11	39.36	0.86	0.001	0.001	0.310	0.746

cAA, crystalline amino acids; CP, crude protein; d, day; N, nitrogen; SE, standard error.

[1]Means of six pens.

[2]Days (d) refer to days posthatching (a common diet was fed from d 1 to 7 posthatching).

[3]P-value of the main effect.

[4]P-value of the contrast 'Diet 3A vs Diets 3B, 3C, 3D, and 3E.'

[5]P-value of the linear contrast of Diets 3B, 3C, 3D, and 3E.

[6]P-value of the quadratic contrast of Diets 3B, 3C, 3D, and 3E.

[7]Whole-body fat content was predicted from the whole-body dry matter content (see text).

[8]Analyzed dietary N used.

[9]Efficiency of N use (N retention divided by N intake).

[10]Protein efficiency ratio (gain divided by CP intake).

[11]Apparent N excretion (N intake – N retention).

[12]N excretion as a percentage of N intake.

19) nor whole-body composition (Table 20) of chicks fed low-CP diets, in accordance with the research of Fancher and Jensen (1989a,c). If, as hypothesized in Experiment 1, free amino acids are metabolized to a larger extent than peptides in the enterocytes, the capacity for metabolism of free amino acids in the enterocyte is apparently sufficiently large to metabolize the relatively high free amino acid concentrations found in Diet 4E.

Chicks fed a 'balanced diet' tend to eat to satisfy their energy requirement rather than their nutrient requirement (Scott et al., 1982; NRC, 1994). In Experiment 3, the ME intake among chicks fed any of the five diets did not differ ($P > 0.10$) regardless of the dietary amino acid content or molecular form (Table 21). This lack of effect would indicate that all the diets were 'balanced' and contained no gross nutrient deficiencies (Scott et al., 1982). The intake of true digestible amino acids supplied in the diet in crystalline form (i.e., arginine, isoleucine, lysine, methionine, threonine, and valine), increased linearly ($P < 0.06$) as their dietary content increased (Table 21). The other amino acids (i.e., from intact protein) were held constant in the four low-CP diets. Their intake (on a true digestible basis) decreased linearly ($P < 0.05$) as higher concentrations of crystalline amino acids were included in the diets, caused by the linear ($P < 0.05$) decrease in ADFI (Table 19). Although statistically significant, the incrementally lower intake of true digestible amino acids from intact protein was numerically small in Diets 3B through 3E (no more than 32 mg/d, corresponding to less than 0.5 g in the 2 wk the treatment diets were fed). Considering that the intake of true digestible amino acids was above the concentration recommended by the NRC (1994; Table 21), the lower amino acid intake was not likely to impact performance.

It was hypothesized that crystalline amino acids were not 100% available for protein synthesis (protein deposition) and that higher dietary inclusion levels would compensate. However, no benefits of adding up to 45% additional crystalline amino acids were found in this experiment. On the contrary, trends ($0.05 < P < 0.10$) suggested that whole-body carcass

Table 21. Nutrient and energy intake[1] calculated from data in Tables 9 and 19 (Experiment 3).

Item[2]	NRC (1994)[3]	Diet 3A Control	Diet 3B Low-CP	Diet 3C Diet 3B + 15% cAA	Diet 3D Diet 3B + 30% cAA	Diet 3E Diet 3B + 45% cAA	SE	P[4]	Contrasts		
									CP[5]	L[6]	Q[7]
Intake (d 7–21)											
ME, kcal/d	225.50	226.31	224.96	224.63	227.88	224.46	2.55	0.860	0.773	0.878	0.548
CP (total), g/d	16.21	15.76	13.34	13.21	13.16	13.01	0.12	0.001	0.001	0.063	0.971
Combined average daily intake of true digestible amino acids from intact protein and crystalline amino acids, mg/d											
Arginine[8]	823	965	843	848	858	862	8	0.001	0.001	0.053	0.939
Cysteine	–	228	190	188	186	184	2	0.001	0.001	0.016	0.843
Glycine	–	617	482	476	472	466	4	0.001	0.001	0.016	0.985
Glycine + serine	823	1265	986	974	967	954	9	0.001	0.001	0.017	0.992
Histidine	230	365	293	289	288	284	3	0.001	0.001	0.015	0.999
Isoleucine[8]	527	611	540	543	550	553	5	0.001	0.001	0.037	0.986
Leucine	753	1143	946	966	927	915	8	0.001	0.001	0.015	0.999
Lysine[8]	724	811	742	752	767	777	7	0.001	0.001	0.001	0.921
Methionine[8]	329	351	417	446	477	504	4	0.001	0.001	0.001	0.797
Methionine[8] + cysteine	593	579	607	634	663	688	6	0.001	0.001	0.001	0.882
Threonine[8]	527	515	540	553	570	582	5	0.001	0.001	0.001	0.917
Tryptophan	132	181	135	133	132	131	1	0.001	0.001	0.021	0.999
Tyrosine	–	517	402	397	394	389	4	0.001	0.001	0.018	0.999
Tyrosine + phenylalanine	882	1219	950	938	932	919	9	0.001	0.001	0.017	0.985
Valine[8]	593	674	607	610	618	620	5	0.001	0.001	0.061	0.952

cAA, crystalline amino acids; CP, crude protein; d, days; ME, metabolizable energy; NRC, National Research Council; SE, standard error; –, no requirement listed by the NRC (1994).

[1]Means of six pens.

[2]Overall period (i.e., d 7 to 21 posthatching). A common diet was fed from d 1 to 7 posthatching.

[3]Calculated from ME and CP levels recommended by the NRC (1994) and the true digestible amino acid requirement listed in Table 9. Feed intake was set at 70.5 g/d, the average of the actual intake in all diets from d 7 to 21 in Experiment 3.

[4]P-value of the main effect.

[5]P-value of the contrast 'Diet 3A vs Diets 3B, 3C, 3D, and 3E.'

[6]P-value of the linear contrast of Diets 3B, 3C, 3D, and 3E.

[7]P-value of the quadratic contrast of Diets 3B, 3C, 3D, and 3E.

[8]Supplied as a combination of intact protein and crystalline amino acids (see Tables 8 and 9).

composition deteriorated with higher dietary concentrations of crystalline amino acids (Table 20): Total amount of whole-body CP and N, as well as N retention, tended ($P < 0.10$) to decrease as the concentration of crystalline amino acids increased. However, at least some of these responses can be attributed to a trend toward a linear ($P = 0.06$) decrease in N intake and a linear ($P < 0.05$) decrease in feed intake.

Experiment 4

Experiment 4 was designed to investigate if dietary non-specific N was limiting growth performance and N retention of broiler chicks fed low-CP diets and if additions of crystalline nonessential amino acids would compensate. A second objective in Experiment 4 was to investigate if a combination of too few nonessential amino acids and a poor utilization of crystalline (essential) amino acids were responsible for the inferior growth performance and N retention of chicks fed low-CP diets. The average room temperature was 24.4 ± 0.4°C from d 7 to 21 posthatching (i.e., during the time when the treatment diets were fed). Three chicks (two chicks fed Diet 4A and one chick fed Diet 4D) died of reasons considered unrelated to the dietary treatments between d 7 and 21 posthatching. Additionally, one chick (fed Diet 4A) was culled due to failure to gain weight and another (fed Diet 4B) was culled because of failure to maintain its equilibrium.

During the first 7 d of feeding the treatment diets, there were no differences ($P > 0.05$) in ADG between the control and the four low-CP diets (i.e., Diets 4B, 4C, 4D, and 4E; Table 22). However, ADFI for chicks fed the control diet was higher ($P < 0.01$), leading to a poorer feed utilization by chicks fed the low-CP diets ($P < 0.001$). The opposite was found from d 14 to 21: Control-fed chicks gained weight significantly faster than chicks fed the low-CP diets, and with no treatment differences in ADFI, feed was utilized less efficiently by the chicks fed low-CP diets ($P < 0.001$). Although the ADG and ADFI in the overall period (d 7 to 21) were not

Table 22. Growth performance[1] (Experiment 4).

Item[2]	Diet 4A Control	Diet 4B Low-CP	Diet 4C Diet 4B + 1% NEAA	Diet 4D Diet 4B + 2% NEAA	Diet 4E Diet 4B + 3% NEAA	Diet 4F Diet 4D + 45% cAA	SE	P[3]	Contrasts				
									CP[4]	L[5]	Q[6]	4A vs 4E[7]	4A vs 4F[8]
Average daily gain, g/d													
d 7–14	29.72	28.78	29.29	29.77	29.80	29.42	0.52	0.731	0.602	0.143	0.648	0.916	0.691
d 14–21	58.02	56.42	55.74	55.16	55.40	55.01	0.82	0.124	0.016	0.325	0.580	0.031	0.014
d 7–21	43.87	42.60	42.52	42.47	42.60	42.21	0.62	0.504	0.067	0.981	0.864	0.158	0.070
Average daily feed intake, g/d													
d 7–14	39.03	40.60	40.35	41.16	41.00	39.67	0.50	0.043	0.004	0.383	0.927	0.010	0.376
d 14–21	77.49	79.19	78.49	78.22	76.92	76.01	1.07	0.355	0.557	0.150	0.779	0.707	0.337
d 7–21	58.26	59.90	59.42	59.69	58.96	57.84	0.76	0.346	0.156	0.457	0.866	0.521	0.697
Feed utilization (gain-to-feed ratio), g/kg													
d 7–14	761	709	726	723	727	742	6	0.001	0.001	0.061	0.264	0.001	0.029
d 14–21	749	712	710	705	720	724	5	0.001	0.001	0.368	0.072	0.001	0.001
d 7–21	753	711	716	712	723	730	4	0.001	0.001	0.074	0.380	0.001	0.001

cAA, crystalline essential amino acids; CP, crude protein; d, day; NEAA, nonessential amino acids; SE, standard error.

[1] Means of six pens.

[2] Days (d) refer to days posthatching (a common diet was fed from d 1 to 7 posthatching).

[3] P-value of the main effect.

[4] P-value of the contrast 'Diet 4A vs Diets 4B, 4C, 4D, and 3E. Note that Diet 4F was not included in this comparison (see text).

[5] P-value of the linear contrast of Diets 4B, 4C, 4D, and 4E. Note that Diets 4A and 4F were not included in this comparison (see text).

[6] P-value of the quadratic contrast of Diets 4B, 4C, 4D, and 4E. Note that Diets 4A and 4F were not included in this comparison (see text).

[7] P-value of the contrast 'Diet 4A vs Diet 4E.'

[8] P-value of the contrast 'Diet 4A vs Diet 4F.'

significantly different between the two groups, chicks fed the low-CP diets utilized feed less efficiently when compared with chicks fed the control diet. Thus, ADG and ADFI varied some from week to week, but chicks fed the control diet consistently utilized feed more efficiently ($P < 0.001$) than the chicks fed any of the four low-CP diets. There were no significant linear or quadratic effects of increasing the concentrations of nonessential amino acids on ADG or ADFI (Table 22). Feed utilization, however, tended ($P = 0.07$) to improve from d 7 to 21 as the concentrations of nonessential dietary amino acids increased, yet not to levels found with the control diet ($P < 0.001$). With an effect of dietary CP concentration, but no linear effects of adding nonessential amino acids on growth performance, even the 'best' low-CP diets (i.e., the one with the most nonessential amino acids added, Diet 4E) resulted in an inferior ($P < 0.05$) feed utilization as compared with the control diet (Diet 4A) throughout the trial.

As in the other experiments, a lower ADG from d 14 to 21 ($P < 0.05$; Table 22) led to a slightly lower ($P = 0.06$) BW on d 21 posthatching in chicks fed the low-CP diets (Diet 4B, 4C, 4D, and 4E) compared with chicks fed the control diet (Table 23). The DM contents of the whole bodies were higher ($P < 0.001$) after feeding the low-CP diets, but decreased linearly ($P < 0.05$) as the concentration of dietary nonessential amino acids increased. Because the whole-body content of fat was estimated from the percentage of whole-body DM (Velu et al., 1972), both the percentage and total amount of fat followed the trend of percent DM, thus improving ($P < 0.05$) whole-body composition with the increase in dietary CP. These effects of nonessential amino acids supplementation were also observed by Aletor et al. (2000). In contrast to the study by Aletor et al. (2000), the percent DM and whole-body fat content in the Experiment 4 did not reach the numerical equivalents of the control diet with the addition of 3% dietary nonessential amino acids in Diet 4E ($P < 0.001$; Table 23). Differences between the control diet and the highest concentration of nonessential amino acid supplementation were estimated with the

Table 23. Body weights and whole-body composition[1] (Experiment 4).

Item[2]	Diet 4A Control	Diet 4B Low-CP	Diet 4C Diet 4B + 1% NEAA	Diet 4D Diet 4B + 2% NEAA	Diet 4E Diet 4B + 3% NEAA	Diet 4F Diet 4D + 45% cAA	SE	P[3]	Contrasts CP[4]	L[5]	Q[6]	4A vs 4E[7]	4A vs 4F[8]
Initial weight (d 7), g	124.17	122.25	122.53	123.18	123.80	121.72	9.21	0.409	0.244	0.208	0.858	0.780	0.070
End weight (d 21), g	738.31	718.69	717.77	717.72	720.15	712.72	8.90	0.435	0.057	0.914	0.852	0.159	0.051
Whole-body content (d 21)													
Dry matter, %	27.28	30.61	29.68	29.45	29.55	29.00	0.35	0.001	0.001	0.044	0.163	0.001	0.002
Fat,[9] %	5.23	9.65	8.41	8.11	8.24	7.51	0.48	0.001	0.001	0.044	0.163	0.001	0.002
Fat,[9] g	38.04	70.09	60.91	58.68	58.82	53.76	3.75	0.001	0.001	0.040	0.224	0.001	0.006
Crude protein, %	16.60	15.71	15.90	15.93	16.07	16.31	0.15	0.004	0.001	0.117	0.854	0.019	0.185
Crude protein, g	122.64	112.87	114.10	114.29	115.71	116.17	1.88	0.015	0.001	0.309	0.963	0.014	0.021
Nitrogen retention													
Whole-body N (d 7), g	2.96	2.91	2.92	2.93	2.95	2.90	0.02	0.412	0.245	0.208	0.862	0.782	0.071
Whole-body N (d 21), g	19.62	18.06	18.27	18.29	18.51	18.59	0.30	0.015	0.001	0.309	0.962	0.014	0.021
N retention (d 7–21), g	16.67	15.15	15.34	15.35	15.57	15.69	0.29	0.015	0.001	0.344	0.779	0.013	0.026
Nitrogen utilization													
N intake,[10] g	30.50	24.50	24.91	25.98	26.45	26.26	0.34	0.001	0.001	0.001	0.935	0.001	0.001
Efficiency,[11] %	54.57	61.80	61.59	59.09	58.87	59.75	0.63	0.001	0.001	0.001	0.993	0.001	0.001
PER[12] (d 7–21), g/g	3.22	3.89	3.82	3.66	3.61	3.60	0.19	0.001	0.001	0.001	0.651	0.001	0.001
N excretion,[13] g	13.84	9.35	9.57	10.63	10.88	10.58	0.19	0.001	0.001	0.001	0.926	0.001	0.001
N excretion,[14] %	45.43	38.19	38.41	40.91	41.13	40.25	0.63	0.001	0.001	0.001	0.994	0.001	0.001

cAA, crystalline essential amino acids; CP, crude protein; d, day; NEAA, nonessential amino acids; SE, standard error.

[1]Means of six pens.

[2]Days (d) refer to days posthatching (a common diet was fed from d 1 to 7 posthatching).

[3]P-value of the main effect.

[4]P-value of the contrast 'Diet 4A vs Diets 4B, 4C, 4D, and 3E.' Note that Diet 4F was not included in this comparison (see text).

[5]P-value of the linear contrast of Diets 4B, 4C, 4D, and 4E. Note that Diets 4A and 4F were not included in this comparison (see text).

[6]P-value of the quadratic contrast of Diets 4B, 4C, 4D, and 4E. Note that Diets 4A and 4F were not included in this comparison (see text).

[7]P-value of the contrast 'Diet 4A vs Diet 4E.'

[8]P-value of the contrast 'Diet 4A vs Diet 4F.'

[9]Whole-body fat content was predicted from the whole-body dry matter content (Velu et al., 1972).

[10]Analyzed dietary N used.

[11]Efficiency of N use (N retention divided by N intake).

[12]Protein efficiency ratio (gain divided by CP intake).

[13]Apparent N excretion (N intake – N retention).

[14]N excretion as a percentage of N intake.

contrast 'Diet 4A vs Diet 4E' for percent whole-body DM, percent whole-body fat, and total whole-body fat content. In each case, Diet 4A was found to be superior ($P < 0.05$).

In contrast to Experiments 1 and 2, but in line with Experiment 3, chicks fed the positive control diet, Diet 4A, had both a higher percentage and total amount of whole-body CP than did chicks fed the low-CP diets ($P < 0.001$; Table 23). Therefore, the carcass composition was significantly affected by the dietary treatments, but was not improved ($P > 0.10$) by the increase in dietary CP through supplementation with nonessential crystalline amino acids. The contrast 'Diet 4A vs Diet 4E' showed that, although percent whole-body CP and total amount of whole-body CP in Diet 4E approximated that of Diet 1A, both were significantly different. It is possible that whole-body composition would eventually match that of Diet 1A with dietary additions of more than 3% crystalline nonessential amino acids. However, efficiency of N use would likely decrease with a concomitant increase in (apparent) N excretion as evidenced by a linear ($P < 0.001$) decrease in N utilization and PER with increasing dietary concentrations of nonessential amino acids.

In Experiments 1 and 3, N retention of chicks fed low-CP diets was lower than that of chicks fed the control diets. This difference was attributed, in part, to a lower BW; however, in this experiment, only a trend ($P = 0.06$) towards lower BW was observed. Nevertheless, the numerically lower BW combined with a lower ($P < 0.001$) whole-body N content (Table 23) led to a lower ($P < 0.001$) N retention than did the control diet, Diet 4A. The content of whole-body N and N retention did not ($P > 0.10$) increase linearly or quadratically as the dietary nonessential amino acid concentrations increased. Whole-body N on d 7 did not ($P > 0.10$) influence the N retention in spite of numerical increases ($P > 0.10$) with increasing concentrations of dietary nonessential amino acids. The data from Experiment 4 indicate that, even with 20% dietary CP (Diet 4E), growth performance suffered in broiler chicks as compared with broiler chicks fed a

23% CP diet (Diet 4A). It is, however, possible that performance would not differ had the CP in diet 4E been from intact protein and not crystalline amino acids as suggested by the results of Experiment 1.

Experiment 4 was designed, in part, to investigate whether the lack of response to low-CP diets in Experiments 1, 2, and 3 were due to a deficiency of nonspecific N (i.e., nonessential amino acids). Although higher concentrations of CP originating from crystalline nonessential amino acids caused a linear ($P < 0.05$) decrease in whole-body fat content, the lack of effects ($P > 0.10$) on growth performance, whole-body CP content, and N retention indicated that the diets in Experiments 2 and 3 contained sufficient nonessential N. This conclusion was supported by the linear ($P < 0.001$) increase in apparent N excretion as dietary nonessential amino acids increased. Evidently, the additional nonessential N was excreted rather than retained by the chicks.

A sixth diet (Diet 4F) was included in Experiment 4 to investigate whether a combination of low utilization of crystalline amino acids and insufficient nonessential N was responsible for the low performance of chicks fed low-CP diets. Adding a combination of essential amino acids (above that needed to meet NRC [1994] recommended concentrations) and nonessential amino acids (i.e., Diet 4F) did not alleviate the poor growth performance (especially the feed utilization) observed in the low-CP diets (Table 22). Similarly, whole-body composition and N retention (Table 23) were inferior ($P < 0.05$) in chicks fed Diet 4F compared with chicks fed the control diet, Diet 4A.

General discussion

In summary, the low-CP diets in all four experiments resulted in inferior growth performance and carcass quality as compared with those from the control diets. These findings corroborate other studies of low-CP diets (e.g., Fancher and Jensen, 1989a,b,c; Pinchasov et al.,

1990; Aletor et al., 2000; Leeson et al., 2000). The poorer performance resulting from the low-CP diets occurred despite attempts to improve the growth performance and N retention through additions of glutamine and asparagine (Experiment 2), as well as crystalline nonessential amino acids (Experiment 4). Dietary additions of crystalline essential amino acids added above what was needed to meet the NRC (1994) recommended concentrations (Experiment 3) did not improve performance either, nor did a combination of increased dietary concentrations of crystalline essential and nonessential amino acids (Experiment 4). Several potential reasons for the inferior growth performance of chicks fed low-CP diets are discussed below.

Dietary amino acid concentrations

The NRC (1994) requirements for total amino acids may be too low for the fast-growing strain of chicks used in the experiments. Moreover, because the amino acid concentrations of the experimental diets were based on NRC (1994) concentrations appropriate for a 50:50 mix of female and male chicks, amino acid concentrations may have been too low for the male chicks used in these experiments (Han and Baker, 1993, 1994). However, the NRC (1994) states that there seems to be little differences in nutrient requirements between male and female broiler chicks when expressed as a percentage of the diet, which was also indicated by Han and Baker (1994). In Experiments 3 and 4, the dietary amino acid concentrations were set at 105% of the concentrations recommended by the NRC (1994), which should have—at least in part—addressed the concerns of too low dietary amino acids concnetrations given the gender and fast-growing strain of the chicks. Additionally, in Experiments 3 and 4, the concentrations of crystalline amino acids were increased above what was needed to meet 105% of the NRC (1994) recommended concentrations. If it is assumed that crystalline (free) amino acids are no different from amino acids originating from intact protein (apart from differences in true digestibility coefficients), a potentially too low NRC (1994) concentration of essential amino acids should

have been alleviated by the increases in crystalline amino acids. Yet, these increases did not improve performance in chicks fed low-CP diets. Furthermore, Diets 1A and 1B contained equal amounts of all amino acids (albeit in different molecular forms), but still resulted in different growth performance and total amount of whole-body CP.

In Experiments 3 and 4, corn and SBM were used to meet the NRC (1994) requirement for tryptophan. Crystalline amino acids were subsequently added to meet or exceed the requirements for the otherwise deficient arginine, isoleucine, lysine, methionine, threonine, and valine. Increasing the concentration of the crystalline amino acids (as was done in Experiments 3 and 4) would not be effective if the NRC (1994) listed requirement for tryptophan was too low—tryptophan would still be limiting growth as it was not added in crystalline form. Han et al. (1991) determined the total tryptophan requirement of 2- to 3-wk-old male broiler chicks to be 0.22% of the diet. The NRC (1994) lists 0.20% as the tryptophan requirement; the diets contained 0.21% total tryptophan. Concentrations of true digestible tryptophan in the low-CP diets in Experiments 3 and 4 corresponded to 0.19% tryptophan, whereas Han et al. (1991) estimated the requirement of digestible tryptophan to 0.20%. Therefore, tryptophan may have been slightly limiting in the low-CP diets (i.e., 5% below the recommended concentration), while not limiting in the control diets. The potentially deficient tryptophan concentrations in the low-CP diets do not, however, explain the differences in performance of chicks fed Diets 1A and 1B, which were formulated to contain equal amounts of all amino acids, including tryptophan.

Growth factors in intact-protein sources

SBM is partially replaced with crystalline amino acids and corn in low-CP diets. Potentially, growth factors in SBM, not present in corn and the mix of crystalline amino acids, may be responsible for the lower performance of chicks fed the low-CP diets. Conceivable growth

factors in SBM include polyamines, saponins, and estrogenic compounds such as isoflavones (Bau et al., 2000). Colnago and Jensen (1992) found no effects of supplementing low-CP diets with the polyamine putrescine on growth performance of broiler chicks. Dietary supplementation with saponins improved growth performance of broilers (Johnston et al., 1981, 1982) and finishing pigs (Mader and Brumm, 1987), and supplementation with isoflavones increased ADG and carcass muscle content in young pigs (Cook, 1998; Greiner, 1999). However, partial replacement of whey protein concentrate (a semi-purified source of intact protein) with crystalline amino acids in an ideal balance decreased ADG and feed utilization in weanling pigs (Davis et al., 1997; de Rodas et al., 1997; Chung et al., 1999). Furthermore, Metges et al. (2000) replaced dietary casein with crystalline amino acids (simulating the amino acid balance of casein) and found significant differences in whole-body net protein synthesis in humans. Thus, it is unlikely that a reduced dietary amount of potential growth factors found in SBM (or other ingredients supplying intact protein) is responsible for the poor performance of animals fed low-CP diets.

Net energy

It has been suggested that low-CP diets result in poor performance because of a relatively high dietary NE content in the low-CP diets. The theory behind this is that chicks tend to eat to satisfy their energy requirements (Scott et al., 1982; NRC, 1994) and diets usually are formulated on an ME basis rather than an NE basis: If two diets—a low-CP and a high-CP diet—are formulated to contain equal amounts of ME and to meet the NRC (1994) recommended concentrations of essential amino acids, the low-CP diet will contain slightly more NE. This is because corn, which replaces SBM in the low-CP diet, has a higher NE content than SBM (De Groote, 1974; NRC, 1998). If chicks eat to satisfy their ME requirement only, the two diets will result in equal intakes of ME, amino acids, and feed. However, the NE intake will be higher in chicks fed the low-CP diets, potentially causing the fatter carcass. More likely, chicks eat to

satisfy their NE requirement, in which case the NE intakes will be similar among chicks fed the two diets. However, ME, amino acid, and feed intakes will differ: Chicks fed the low-CP diet will eat less feed and consequently consume fewer grams of amino acids, compromising growth performance and/or the protein deposition capability. Even though the experiments reported herein were not designed to investigate potential effects of the dietary NE concentration, the dietary NE concentrations were calculated using NE values of corn, SBM, soybean oil, L-lysine·HCl, and DL-methionine reported by De Groote (1974). Because of a lack of reported NE values for cornstarch, the NE value was set at 90% of the ME_n value used, corresponding to 3312 kcal NE/kg. The NE values of crystalline amino acids (other than lysine and methionine) were set at 60% (De Groote, 1974) of their respective ME_n values reported by the NRC (1994). The NE value of TAC (Experiment 2) was assumed to equal that of glutamine. ME and NE intakes were subsequently calculated from the dietary ME and NE contents and the overall feed intakes (i.e., the ADFI from d 7 through d 21 posthatching). Results from these calculations are shown in Tables 24, 25, 26, and 27 for Experiments 1, 2, 3, and 4, respectively.

Table 24. Estimated metabolizable and net energy intakes,[1] d 14–21 (Experiment 1).

Item[2]	Diet 1A Intact protein (positive control)	Diet 1B Crystalline essential and nonessential amino acids	Diet 1C Crystalline essential amino acids	Diet 1D Low-CP (negative control)	SE	P[3]
Dietary content, kcal/kg						
ME	3197.65	3199.24	3198.16	3195.27	–	–
NE	2428.12	2528.18	2557.89	2544.52	–	–
Average daily intake (d 7–21)						
Feed, g/d	58.43[a]	53.63[b]	51.00[c]	57.11[a]	0.85	0.001
ME, kcal/d	186.86[a]	171.58[b]	163.11[c]	182.46[a]	2.71	0.001
NE, kcal/d	141.89[ac]	135.59[ac]	130.45[c]	145.31[b]	2.15	0.001

d, day; CP, crude protein; ME, metabolizable energy; NE, net energy; SE, standard error; –, not applicable.
[1] Least-squares means (n = 6).
[2] Days (d) refer to days posthatching (a common diet was fed from d 1 to 7 posthatching).
[3] P-value of the main effect.
[abc] Means within a row lacking a common superscript differ (P < 0.05): estimated by Fischer's protected least significant difference.

Table 25. Estimated metabolizable and net energy intakes,[1] d 14–21 (Experiment 2).

Item[2]	Diet 2A Control	Diet 2B Low-CP	Diet 2C Diet 2B + 1% Gln	Diet 2D Diet 2B + 1% Asn	SE	P[3]	Contrast[4]
Dietary content, kcal/kg							
ME	3200.29	3200.29	3200.29	3191.68	–	–	–
NE	2431.45	2428.22	2428.22	2423.05	–	–	–
Average daily intake (d 7–21)							
Feed, g/d	63.23	65.80	65.20	64.82	0.64	0.083	0.019
ME, kcal/d	149.85[a]	144.21[b]	141.80[b]	141.95[b]	1.60	0.006	0.001
NE, kcal/d	113.85[a]	109.45[b]	107.59[b]	107.77[b]	1.21	0.005	0.001

Asn, asparagine; CP, crude protein; d, day; Gln, glutamine; ME, metabolizable energy; NE, net energy; SE, standard error; –, not applicable.

[1]Least-squares means (n = 6).

[2]Days (d) refer to days posthatching (a common diet was fed from d 1 to 7 posthatching).

[3]P-value of the main effect.

[4]P-value of the contrast 'Diet 2A vs Diets 2B, 2C, and 2D.'

[a,b]Means within a row lacking a common superscript differ ($P < 0.05$), estimated by Fischer's protected least significant difference.

Neither ME nor NE intake differed ($P > 0.10$) among the control diet and the low-CP diet (Diet 1D) in Experiment 1 (Table 24), Experiment 3 (Table 26), or Experiment 4 (Table 27). Even the relatively large difference in dietary NE content in Experiment 1 (116 kcal/kg; Table 24) did not result in any differences in daily energy intake between the low-CP (Diet 1D) and the control diet (Diet 1A). Thus, superior performance of chicks fed the control diets cannot be attributed to a higher or lower intake of ME or NE. This conclusion is parallel with the findings of Knowles et al. (1998) and Leeson et al. (2000), who did not detect any influences of dietary NE content on performance of pigs and chicks fed low-CP diets. Only in Experiment 2 did a small difference in dietary NE content (8 kcal/kg) produce a significant difference in energy intake between chicks fed the control diet and chicks fed the low-CP diets (Table 25). However, this difference was evident in both ME and NE intake and was, although highly significant, numerically small. The intakes of ME and NE were lower in chicks fed the low-CP diets compared with the respective energy intakes of chicks fed the control diet, suggesting that other dietary factors are to blame for the lower feed intake. It should be noted that the dietary energy contents

Table 26. Estimated metabolizable and net energy intakes,[1] d 14–21 (Experiment 3).

Item[2]	*Diet 3A* Control	*Diet 3B* Low-CP	*Diet 3C* Diet 3B + 15% cAA	*Diet 3D* Diet 3B + 30% cAA	*Diet 3E* Diet 3B + 45% cAA	SE	P[3]	Contrasts		
								CP[4]	L[5]	Q[6]
Dietary content, kcal/kg										
ME	3199.96	3200.22	3201.81	3203.34	3204.75	–	–	–	–	–
NE	2432.73	2427.91	2428.60	2429.26	2429.85	–	–	–	–	–
Average daily intake (d 7–21)										
Feed, g/d	68.57	72.11	71.18	70.72	69.77	0.61	0.004	0.002	0.011	0.987
ME, kcal/d	226.31	224.96	224.63	227.88	224.46	2.55	0.860	0.773	0.878	0.548
NE, kcal/d	172.05	170.67	170.38	172.81	170.18	1.93	0.873	0.635	0.910	0.549

cAA, crystalline amino acids; CP, crude protein; d, day; ME, metabolizable energy; NE, net energy; SE, standard error; –, not applicable.

[1]Least-squares means, n = 6.

[2]Days (d) refer to days posthatching (a common diet was fed from d 1 to 7 posthatching).

[3]P-value of the main effect.

[4]P-value of the contrast 'Diet 3A vs Diets 3B, 3C, 3D, and 3E.'

[5]P-value of the linear contrast of Diets 3B, 3C, 3D, and 3E.

[6]P-value of the quadratic contrast of Diets 3B, 3C, 3D, and 3E.

Table 27. Estimated metabolizable and net energy intakes,[1] d 14–21 (Experiment 4).

Item[2]	Diet 4A Control	Diet 4B Low-CP	Diet 4C Diet 4B + 1% NEAA	Diet 4D Diet 4B + 2% NEAA	Diet 4E Diet 4B + 3% NEAA	Diet 4F Diet 4D + 45% cAA	SE	P[3]	Contrasts				
									CP[4]	L[5]	Q[6]	4A vs 4E[7]	4A vs 4F[8]
Dietary content, kcal/kg													
ME	3199.80	3199.99	3200.26	3200.13	3199.93	3200.74	-	-	-	-	-	-	-
NE	2435.37	2452.25	2446.78	2440.95	2435.05	2437.32	-	-	-	-	-	-	-
Average daily intake (d 7–21)													
Feed, g/d	58.26	59.90	59.42	59.69	58.96	57.84	0.76	0.346	0.156	0.457	0.866	0.521	0.697
ME, kcal/d	140.37	136.33	136.06	135.90	136.61	135.12	1.99	0.510	0.068	0.979	0.867	0.159	0.072
NE, kcal/d	106.83	104.47	104.03	103.66	103.72	102.89	1.51	0.557	0.102	0.703	0.868	0.158	0.076

cAA, crystalline essential amino acids; CP, crude protein; d, day; ME, metabolizable energy; NE net energy; NEAA, nonessential amino acids; SE, standard error; –, not applicable.

[1]Least-squares means, n = 6.

[2]Days (d) refer to days posthatching (a common diet was fed from d 1 to 7 posthatching).

[3]P-value of the main effect.

[4]P-value of the contrast 'Diet 4A vs Diets 4B, 4C, 4D, and 3E.' Note that Diet 4F was not included in this comparison (see text).

[5]P-value of the linear contrast of Diets 4B, 4C, 4D, and 4E. Note that Diets 4A and 4F were not included in this comparison (see text).

[6]P-value of the quadratic contrast of Diets 4B, 4C, 4D, and 4E. Note that Diets 4A and 4F were not included in this comparison (see text).

[7]P-value of the contrast 'Diet 4A vs Diet 4E.'

[8]P-value of the contrast 'Diet 4A vs Diet 4F.'

listed in Tables 24, 25, 26, and 27 were calculated from published values. Therefore, the dietary energy contents and energy intakes were not intended to substitute for experiments designed to investigate potential influences of energy intake.

Intact protein versus free amino acids

In Experiment 1, it was hypothesized that free amino acids are not utilized as well for growth and protein synthesis as are amino acids absorbed into the enterocytes in the form of di- and tripeptides. Results from Experiment 1 (Diets 1A vs 1B) indicated that chicks do not grow as fast or utilize feed as efficiently when the majority of the dietary protein is in the form of free amino acids as compared with intact protein. The same was evident from Experiments 2, 3, and 4, in which a large proportion of the dietary protein in the low-CP diets was supplied as free amino acids. In agreement with the low ADG and feed utilization, respectively, BW at the end of the trials was lower in the chicks that had been fed a low-CP diet compared with chicks fed control diets and their carcasses were also fatter. This response would indicate that dietary amino acids were deaminated and their carbon skeletons used to supply energy or, more likely, were stored as triacylglycerols in adipocytes.

Because the carcass fat content was higher, the N retention lower, and energy intake unaffected in chicks fed low-CP diets as compared with those fed the control diets, (at least some of) the additionally stored fat in chicks fed low-CP diets must have come from dietary amino acids. The question then arises whether amino acids from dietary free amino acids are metabolized to a greater extent than dietary amino acids originating from intact protein. Mechanisms would have to be in place to differentiate amino acids based on their origin (i.e., free amino acids vs intact protein) before a preferential catabolism would occur. Differences in the time it takes the dietary amino acids to appear in the portal blood between free (crystalline) amino acids and intact-protein amino acids (Buraczewska et al., 1980; Rérat et al. 1984; Dangin

et al., 2001) may offer such a mechanism. In animals fed once a day, the difference would lead to an imbalanced supply of amino acids in the portal blood (and subsequently to the liver and other extra-intestinal tissues) with increased deamination and catabolism as a result. However, in animals with free access to feed (or fed more than two to three times daily), these differences in the plasma amino acid profile would equilibrate as implied by several investigators (Batterham, 1974; Batterham and O'Neill, 1978; Baker and Izquierdo, 1985; Partridge et al., 1985). However, their aforementioned experiments only incorporated *one* crystalline amino acid, namely lysine, while all other amino acids were supplied from intact protein. Correspondingly, when one amino acid was supplied in free form, whole-body net protein synthesis rate and whole-body amino acid oxidation were not significantly different from those obtained with an all-intact protein diet in a study by Metges et al. (2000). Yet, the net protein synthesis rate and amino acid oxidation were numerically inferior to the intact protein diet, and, in spite of hourly meals, became significantly inferior when all the protein originated from free amino acids. The results of Metges et al. (2000) suggest that adding more than one crystalline amino acid to a diet results in significantly inferior growth performance and N retention regardless of 'frequent feeding.' The conclusions by Batterham (1974), Batterham and O'Neill (1978), Baker and Izquierdo (1985), and Partridge et al. (1985) are therefore only valid when *one* dietary amino acid is supplied in crystalline form (i.e., only a relatively small proportion of the dietary protein is supplied as free amino acids). This would also explain why the dietary CP content can be lowered by two percentage points without affecting growth performance and/or carcass quality (Lewis, 2001) as only one crystalline amino acid (lysine) is used to replace parts of the intact protein in the diets. Data from Experiment 1 do indeed support the study by Metges et al. (2000) in that the net whole-body protein synthesis rate (measured as whole-body lean gain) differed ($P < 0.001$) between chicks fed Diets 1A and 1B (6.67 ± 0.16 and 5.94 ± 0.02 g CP/d, respectively). In contrast, Chung and Baker (1991) found no differences ($P > 0.05$) in

growth performance or N retention between pigs (10 to 20 kg initial BW) fed an intact-protein diet (protein supplied by a combination of corn, SBM, and dried whey) for 25 d and pigs fed a purified diet, in which all the protein was supplied by free (crystalline) amino acids.

The issue of preferential metabolism between dietary free amino acids and amino acids from intact protein could also stem from differences in absorption rates between intact protein and free amino acids. Dietary free amino acids appear sooner in the plasma than amino acids from intact protein due to differences in digestion (hydrolysis) time in the small intestine. Even though frequent feeding should eliminate these differences over time (Batterham, 1974; Batterham and O'Neill, 1978; Baker and Izquierdo, 1985; Partridge et al., 1985), hourly meals of free amino acids resulted in spikes in plasma amino acid concentration that were not observed when hourly meals of intact protein were ingested (Metges et al., 2000). The spikes may lead to plasma amino acid concentrations at which the protein synthesis rate is exceeded, in effect creating a "surplus" of amino acids in the plasma. As no storage mechanisms exist for the surplus amino acids, they are transaminated and their carbon skeletons stored in the body as triacylglycerols. Consequently, plasma amino acids will become deficient (when measured over time), leading to a lower protein deposition (and lean gain).

The different absorption mechanisms between free amino acids and peptides may also provide a mode of action by which the cells differentiate between dietary intact protein and free amino acids. Presumably, the majority of intact protein is absorbed into the enterocytes as di- and tripeptides (Groff and Gropper, 2000), which, upon entrance into the enterocytes, are hydrolyzed to free amino acids by peptidases in the cytosol. Consequently, most amino acids exit the enterocytes through the basolateral membrane as free amino acids (Stevens, 2000). Potentially, these cytosolic peptidases could transport the amino acids originating from the peptides to the basolateral membrane without releasing them into the cytosol (i.e., the peptidase would double as a transport protein). This transport would 'protect' the peptide-bound amino

acids from (partial) oxidation or metabolism in the enterocytes, while no such protection would be offered for amino acids absorbed as free amino acids. If this is true, the low-CP diets would result in inferior performance because fewer amino acids originating from free (crystalline) sources would reach the portal blood and thereby the extra-intestinal tissues. Already, there is a considerable oxidation of amino acids in the enterocytes (Stoll et al., 1998; Wu, 1998). Rérat and colleagues (Rérat, 1985; Rérat et al., 1984, 1988, 1992) showed that the absorption of amino acids into the portal blood is greater if they originate from low-molecular weight peptides than if they originate from combinations of free amino acids of similar amino acid composition as the peptides, supporting this theory.

Energetic cost of amino acid absorption

It may be more efficient to absorb di- and (especially) tripeptides than free amino acids. The ATP cost of absorbing one free amino acid, one dipeptide, and one tripeptide into the enterocyte is similar; however, more amino acids enter the enterocytes per ATP expended when peptides are absorbed. If it is accepted that the majority of dietary (intact) protein is absorbed into the enterocytes as di- and tripeptides, the energetic cost of absorbing the dietary protein will be higher in low-CP diets due to their high content of free (crystalline) amino acids compared with the protein from diets based on intact protein. This relatively higher ATP expenditure could account for the poor feed utilization observed in all four experiments when low-CP diets were fed. Furthermore, the higher need for ATP in the enterocytes may come from (partial) catabolism of amino acids (Windmueller and Spaeth, 1980; Stoll et al., 1998, Reeds et al., 2000), thereby decreasing the availability of amino acids in extra-intestinal tissues. The reduced availability, in turn, would account for the low whole-body CP content in chicks fed low-CP diets. The lower availability of amino acids for protein deposition, in effect, increases the energy-to-nutrient (amino acid) ratio, resulting in a 'surplus' of energy in relation to that required for

protein deposition. The 'surplus' energy is instead used for fat deposition, explaining the fatter carcasses of chicks fed low-CP diets.

Conclusion and implications

In conclusion, the low-CP diets in all four experiments led to inferior growth performance, less grams of whole-body CP, and more whole-body fat compared with the high-CP control diets. None of the treatments in Experiment 2, 3, or 4 alleviated the poor performance of broiler chicks fed the low-CP diets; chicks still had inferior growth performance and carcass quality than did chicks fed a 'standard' 23% CP diet. As a result, it is not recommended to feed low-CP diets (i.e., diets with a CP content decreased more than three to four percentage points compared to NRC recommendations, regardless of supplementation with crystalline, free amino acids) to 0- to 3-wk-old broiler chicks. Chicks fed any of the low-CP diets utilized the dietary protein with a higher efficiency, meaning that less N was excreted in the manure. Hence, if minimization of N excretion is valued above that of growth performance and/or carcass quality, low-CP diets can be fed with success.

Although the four experiments reported herein did not pinpoint the cause or causes for the inferior performance resulting from low-CP diets, the results indicated that dietary free amino acids are not utilized as well for protein deposition and lean gain as amino acids from intact protein (i.e., corn and SBM). At least two reasons may be responsible for this and should be the subject of future studies on a cellular or organ basis rather than on a whole-animal basis. The first is that the energetic cost of absorbing free amino acids may be higher than the cost of absorbing di- and tripeptides into the enterocytes. To validate this, it must be established experimentally that the majority of dietary protein from intact protein is absorbed into the enterocytes as di- and tripeptides. Second, there may be a preferential (partial) catabolism of dietary free amino acids as compared with amino acids originating from intact protein (i.e.,

di- and tripeptides). The catabolism may arise from differences in metabolism of di- and tripeptides and free amino acids in the enterocytes, potentially through a protection of di- and tripeptides within the enterocytes. Additionally, the preferential catabolism may stem from spikes in plasma amino acid concentrations, originating from differences in absorption rate between dietary free amino acids and intact protein. These spikes may, in turn, lead to plasma amino acid concentrations at which the capacity for protein synthesis is exceeded, leading to an increased oxidation of dietary free amino acids. The potentially higher energetic costs of absorbing free amino acids and the preferential catabolism of dietary free amino acids are not mutually exclusive; a combination of the two may be responsible for the poor performance of growing chicks and pigs fed low-CP diets.

APPENDIX

ESTIMATION OF THE TRUE DIGESTIBLE AMINO ACID CONTENTS FROM THE ANALYZED TOTAL AMINO ACID CONTENTS

Dietary values

Analyzed dietary amino acid levels only yield information about total amino acid contents—not the digestible amino acid content. Therefore, an effort was made to calculate the dietary contents of true digestible amino acids from the analyzed total amino acid content using digestibility values for corn and SBM listed by the NRC (1994). Crystalline amino acids were assumed to be 100% digestible (Izquierdo et al., 1988; Chung and Baker, 1992). Lysine and Diet 1B, respectively, are used as examples of an amino acid and a diet in all equations below. The results of the calculations—the estimated concentrations of dietary true digestible amino acids, as well as estimated requirements for true digestible amino acids—are shown in Table 13.

The ratio of corn:SBM was 1.338 (rounded to 1.34 in Table 3) in all diets in Experiment 1, meaning that corn made up 74.7% [3] and SBM 25.3% [4] of the combined dietary corn and SBM content.

$$\text{Proportion of corn} = 1.000 \div 1.338 = 0.747 \quad [3]$$

$$\text{Proportion of soybean meal} = 1.000 - 0.747 = 0.253 \quad [4]$$

The NRC (1994) lists a true digestibility coefficient of 0.81 and 0.91 for lysine in corn and SBM, respectively. Therefore, the combined true digestibility coefficient of lysine in the combined corn and SBM fraction was:

$$(0.81 \times 0.747) + (0.91 \times 0.253) = 0.84 \quad [5]$$

The true digestibility of crystalline lysine was assumed to be 100% (Izquierdo et al., 1988; Chung and Baker, 1992). Thus, the true digestibility coefficient for dietary lysine is 0.84 [5] for the proportion of lysine from intact protein (i.e., the lysine from corn and SBM) and 1.00 for the proportion of lysine from crystalline lysine.

Because it is impossible to distinguish lysine from intact protein and crystalline lysine in the analyzed total amino acid content, the proportion of lysine from crystalline lysine was estimated from the diet-formulation spreadsheet (not shown). In Diet 1B, the sources of crystalline lysine were L-lysine·HCl and Tryptosine™, which were included as 0.07 and 0.95% of the diet, respectively (Table 2). As formulated (not analyzed), the two sources contributed 0.577 percentage points (calculation not shown) of the 1.246% dietary total lysine in the diet (data not shown). Thus, the contribution of dietary lysine from crystalline sources [6] and intact protein was calculated [7].

$$\text{Proportion of lysine from crystalline sources} = 0.577 \div 1.246 = 0.463 \quad [6]$$

$$\text{Proportion of lysine from corn and soybean meal} = 1.000 - 0.463 = 0.537 \quad [7]$$

In other words, 46.3% of the total lysine came from crystalline lysine (which had a true digestibility coefficient of 1.00), whereas 53.7% of the total lysine came from intact protein (which had a true digestibility coefficient of 0.84). The analyzed total lysine in Diet 1B was

1.18% (Table 13); the dietary true digestible lysine from crystalline lysine was subsequently calculated as in [8] using data from [6] and a digestibility coefficient of 1.00 for crystalline lysine.

$$1.18\% \times (1.00 \times 0.463) = 0.55\% \quad [8]$$

Similarly, the dietary true digestible lysine from intact protein was calculated as shown in [9] using data from [5] and [7].

$$1.18\% \times (0.84 \times 0.537) = 0.53\% \quad [9]$$

It follows that the content of true digestible lysine is Diet 1B is 1.08% [10]. Table 13 lists a value of 1.07% true digestible lysine—a slight difference due to rounding.

$$0.53\% \text{ intact-protein lysine} + 0.55\% \text{ crystalline lysine} = 1.08\% \quad [10]$$

The lysine in Diet 1B was thus estimated to be 91.5% true digestible [11].

$$(1.08\% \text{ true digestible lysine} \div 1.18\% \text{ total lysine}) \times 100\% = 91.53\% \quad [11]$$

Requirements

The NRC (1994) does not list a requirement for true digestible amino acids, but rather a requirement for total amino acids based on experiments with amino acids originating from corn and SBM. Therefore, the requirements for true digestible amino acids had to be estimated in order to evaluate the adequacy of Diets 1A, 1B, 1C, and 1D using the same principles as shown above with lysine again serving as an example.

The corn:SBM ratios of the diets that the NRC (1994) amino acid requirements were based upon were unknown and the value of 1.338 was consequently used. This ratio was based on the corn:SBM ratio in the control diet, Diet 1A, which was formulated using corn and SBM (Tables 2 and 3), allowing the estimated requirement for true digestible lysine to be calculated using [3], [4], and [5]. The requirement for total lysine for 0- to 3-wk-old chicks is 1.10% total lysine (NRC, 1994); it follows that the requirement for true digestible lysine is 0.92% [12].

$$0.84 \times 1.10\% \text{ total lysine} = 0.92\% \text{ true digestible lysine} \quad [12]$$

Note: The calculated requirements (Equations [3] through [12] and Table 13) were based on several assumptions and were therefore not meant to substitute for future experimentally-determined estimations of the true digestible lysine (and other amino acids) requirements for broiler chicks.

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